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Interleukin-4 receptors.

Mammalian Interleukin-4 receptor proteins, DNAs and expression vectors encoding mammalian IL-4 receptors, and processes for producing mammalian IL-4 receptors as products of cell culture, are disclosed.

## Interleukin-4 Receptors

The present invention relates generally to cytokine receptors and, more specifically, to Interleukin-4 receptors.

Interleukin-4 (IL-4, also known as B cell stimulating factor, or BSF-1) was originally characterized by its ability to stimulate the proliferation of B cells in response to low concentrations of antibodies directed to surface immunoglobulin. More recently, IL-4 has been shown to possess a far broader spectrum of biological activities, including growth co-stimulation of T cells, mast cells, granulocytes, megakaryocytes, and erythrocytes. In addition, IL-4 stimulates the proliferation of several IL-2- and IL-3-dependent cell lines, induces the expression of class II major histocompatibility complex molecules on resting B cells, and enhances the secretion of IgE and IgG1 isotypes by stimulated B cells. Both murine and human IL-4 have been definitively characterized by recombinant DNA technology and by purification to homogeneity of the natural murine protein (Yokota et al., *Proc. Natl. Acad. Sci. USA* 83:5894, 1986; Noma et al., *Nature* 319:640, 1986; and Grabstein et al., *J. Exp. Med.* 163:1405, 1986).

The biological activities of IL-4 are mediated by specific cell surface receptors for IL-4 which are expressed on primary cells and *in vitro* cell lines of mammalian origin. IL-4 binds to the receptor, which then transduces a biological signal to various immune effector cells. Purified IL-4 receptor (IL-4R) compositions will therefore be useful in diagnostic assays for IL-4 or IL-4 receptor, and in raising antibodies to IL-4 receptor for use in diagnosis or therapy. In addition, purified IL-4 receptor compositions may be used directly in therapy to bind or scavenge IL-4, providing a means for regulating the biological activities of this cytokine.

Although IL-4 has been extensively characterized, little progress has been made in characterizing its receptor. Numerous studies documenting the existence of an IL-4 receptor on a wide range of cell types have been published; however, structural characterization has been limited to estimates of the molecular weight of the protein as determined by SDS-PAGE analysis of covalent complexes formed by chemical cross-linking between the receptor and radiolabeled IL-4 molecules. Ohara et al. (*Nature* 325:537, 1987) and Park et al. (*Proc. Natl. Acad. Sci. USA* 84:1669, 1987) first established the presence of an IL-4 receptor using radioiodinated recombinant murine IL-4 to bind a high affinity receptor expressed in low numbers on B and T lymphocytes and a wide range of cells of the hematopoietic lineage. By affinity cross-linking  $^{125}$ I-IL-4 to IL-4R, Ohara et al. and Park et al. identified receptor proteins having apparent molecular weights of 60,000 and 75,000 daltons, respectively. It is possible that the small receptor size observed on the murine cells represents a proteolytically cleaved fragment of the native receptor. Subsequent experiments by Park et al. (*J. Exp. Med.* 166:476, 1987) using yeast-derived recombinant human IL-4 radiolabeled with  $^{125}$ I showed that human IL-4 receptor is present not only on cell lines of B, T, and hematopoietic cell lineages, but is also found on human fibroblasts and cells of epithelial and endothelial origin. IL-4 receptors have since been shown to be present on other cell lines, including CBA/N splenic B cells (Nakajima et al., *J. Immunol.* 139:774, 1987), Burkitt lymphoma Jijoye cells (Cabrillat et al., *Biochem. & Biophys. Res. Commun.* 149:995, 1987), a wide variety of hemopoietic and nonhemopoietic cells (Lowenthal et al., *J. Immunol.* 140:456, 1988), and murine Lyt-2<sup>+</sup>L3T4<sup>+</sup> thymocytes. More recently, Park et al. (UCLA Symposium, *J. Cell Biol.*, Suppl. 12A, 1988) reported that, in the presence of sufficient protease inhibitors,  $^{125}$ I-IL-4-binding plasma membrane receptors of 138-145 kDa could be identified on several murine cell lines. Considerable controversy thus remains regarding the actual size and structure of IL-4 receptors.

Further study of the structure and biological characteristics of IL-4 receptors and the role played by IL-4 receptors in the responses of various cell populations to IL-4 or other cytokine stimulation, or of the methods of using IL-4 receptors effectively in therapy, diagnosis, or assay, has not been possible because of the difficulty in obtaining sufficient quantities of purified IL-4 receptor. No cell lines have previously been known to express high levels of IL-4 receptors constitutively and continuously, and in cell lines known to express detectable levels of IL-4 receptor, the level of expression is generally limited to less than about 2000 receptors per cell. Thus, efforts to purify the IL-4 receptor molecule for use in biochemical analysis or to clone and express mammalian genes encoding IL-4 receptor have been impeded by lack of purified receptor and a suitable source of receptor mRNA.

The present invention provides DNA sequences encoding mammalian Interleukin-4 receptors (IL-4R) or subunits thereof. Preferably, such DNA sequences are selected from the group consisting of: (a) cDNA clones having a nucleotide sequence derived from the coding region of a native IL-4R gene; (b) DNA sequences capable of hybridization to the cDNA clones of (a) under moderately stringent conditions and which encode biologically active IL-4R molecules; and (c) DNA sequences which are degenerate, as a result of the genetic code, to the DNA sequences defined in (a) and (b) and which encode biologically active IL-

4R molecules. The present invention also provides recombinant expression vectors comprising the DNA sequences defined above, recombinant IL-4R molecules produced using the recombinant expression vectors, and processes for producing the recombinant IL-4R molecules using the expression vectors.

The present invention also provides substantially homogeneous protein compositions comprising mammalian IL-4R. The full length murine molecule is a glycoprotein having a molecular weight of about 130,000 to about 140,000 M, by SDS-PAGE. The apparent binding affinity ( $K_d$ ) for COS cells transfected with murine IL-4 receptor clones 16 and 18 from the CTLL 19.4 library is 1 to  $8 \times 10^9$  M<sup>-1</sup>. The  $K_d$  for COS cells transfected with murine IL-4 receptor clones 7B9-2 and 7B9-4 from the murine 7B9 library is  $2 \times 10^9$  to  $1 \times 10^{10}$  M<sup>-1</sup>. The mature murine IL-4 receptor molecule has an N-terminal amino acid sequence as follows: IKVLGEPTCFSDYIRTSTCEW.

The human IL-4R molecule is believed to have a molecular weight of between about 110,000 and 150,000 M, and has an N-terminal amino acid sequence, predicted from the cDNA sequence and by analogy to the biochemically determined N-terminal sequence of the mature murine protein, as follows: MKVLQEPTCVSDYMSISTCEW.

The present invention also provides compositions for use in therapy, diagnosis, assay of IL-4 receptor, or in raising antibodies to IL-4 receptors, comprising effective quantities of soluble receptor proteins prepared according to the foregoing processes. Such soluble recombinant receptor molecules include truncated proteins wherein regions of the receptor molecule not required for IL-4 binding have been deleted. These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. The invention will now be described by way of example with reference to the accompanying drawings, in which:

Figure 1 shows restriction maps of cDNA clones containing the coding regions (denoted by a bar) of the murine and human IL-4R cDNAs. The restriction site *EcoRI*, *PvuII*, *HincII* and *SstI* are represented by the letters R, P, H and S, respectively.

Figures 2A-C depict the cDNA sequence and the derived amino acid sequence of the coding region of a murine IL-4 receptor, as derived from clone 7B9-2 of the 7B9 library. The N-terminal isoleucine of the mature protein is designated amino acid number 1. The coding region of the full-length membrane-bound protein from clone 7B9-2 is defined by amino acids 1-785. The ATC codon specifying the isoleucine residue constituting the mature N-terminus is underlined at position 1 of the protein sequence; the putative transmembrane region at amino acids 209-232 is also underlined. The sequences of the coding regions of clones 7B9-4 and clones CTLL-18 and CTLL-16 of the CTLL 19.4 library are identical to that of 7B9-2 except as follows. The coding region of CTLL-16 encodes a membrane-bound IL-4-binding receptor defined by amino acids -25 through 233 (including the putative 25 amino acid signal peptide sequence), but is followed by a TAG terminator codon (not shown) which ends the open reading frame. The nucleic acid sequence indicates the presence of a splice donor site at this position (indicated by an arrow in Figure 1) and a splice acceptor site near the 3' end (indicated by a second arrow), suggesting that CTLL-16 was derived from an unspliced mRNA intermediate. Clones 7B9-4 and CTLL-18 encode amino acids 23 through 199 and -25 through 199, respectively. After amino acid 199, a 114-base pair insert (identical in both clones and shown by an open box in Figure 1) introduces six new amino acids, followed by a termination codon. This form of the receptor is soluble.

Figure 3 is a schematic illustration of the mammalian high expression plasmid pCAV/NOT, which is described in greater detail in Example 8.

Figures 4A-C depict the coding sequence of a human IL-4 receptor cDNA from clone T22-8, which was obtained from a cDNA library derived from the T cell line T22. The predicted N-terminal methionine of the mature protein and the transmembrane region are underlined.

Figures 5A-B are a comparison of the predicted amino acid sequences of human (top line) and murine (bottom line) IL-4 receptor cDNA clones.

## Definitions

As used herein, the terms "IL-4 receptor" and "IL-4R" refer to proteins having amino acid sequences which are substantially similar to the native mammalian Interleukin-4 receptor amino acid sequences disclosed in Figures 2 and 4, and which are biologically active as defined below, in that they are capable of binding Interleukin-4 (IL-4) molecules or transducing a biological signal initiated by an IL-4 molecule binding to a cell, or cross-reacting with anti-IL-4R antibodies raised against IL-4R from natural (i.e., nonrecombinant) sources. The native murine IL-4 receptor molecule is thought to have an apparent molecular weight by SDS-PAGE of about 140 kilodaltons (kDa). The terms "IL-4 receptor" or "IL-4R" include, but are not limited

to, analogs or subunits of native proteins having at least 20 amino acids and which exhibit at least some biological activity in common with IL-4R. As used throughout the specification, the term "mature" means a protein expressed in a form lacking a leader sequence as may be present in full-length transcripts of a native gene. Various bioequivalent protein and amino acid analogs are described in detail below.

5 The term "substantially similar," when used to define either amino acid or nucleic acid sequences, means that a particular subject sequence, for example, a mutant sequence, varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which is to retain biological activity of the IL-4R protein. Alternatively, nucleic acid subunits and analogs are "substantially similar" to the specific DNA sequences disclosed herein if: (a) the DNA sequence is derived from the coding region of a native mammalian IL-4R gene; (b) the DNA sequence is capable of hybridization to DNA sequences of (a) under moderately stringent conditions and which encode biologically active IL-4R molecules; or DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) or (b) and which encode biologically active IL-4R molecules. Substantially similar analog proteins may be greater than about 30 percent similar to the corresponding sequence of the native IL-4R. Sequences having lesser degrees of similarity but comparable biological activity are considered to be equivalents. More preferably, the analog proteins will be greater than about 80 percent similar to the corresponding sequence of the native IL-4R, in which case they are defined as being "substantially identical." In defining nucleic acid sequences, all subject nucleic acid sequences capable of encoding substantially similar amino acid sequences are considered substantially similar to a reference nucleic acid sequence. Percent similarity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482, 1981). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, ed., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

"Recombinant," as used herein, means that a protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial" defines a protein produced in a microbial expression system which is essentially free of native endogenous substances. Protein expressed in most bacterial cultures, e.g., *E. coli*, will be free of glycan. Protein expressed in yeast may have a glycosylation pattern different from that expressed in mammalian cells.

"Biologically active," as used throughout the specification as a characteristic of IL-4 receptors, means that a particular molecule shares sufficient amino acid sequence similarity with the embodiments of the present invention disclosed herein to be capable of binding detectable quantities of IL-4, transmitting an IL-4 stimulus to a cell, for example, as a component of a hybrid receptor construct, or cross-reacting with anti-IL-4R antibodies raised against IL-4R from natural (i.e., nonrecombinant) sources. Preferably, biologically active IL-4 receptors within the scope of the present invention are capable of binding greater than 0.1 nmoles IL-4 per nmole receptor, and most preferably, greater than 0.5 nmole IL-4 per nmole receptor in standard binding assays (see below).

45 "DNA sequence" refers to a DNA molecule, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions. The sequences are preferably at least 60 bases long.

55 "Nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit.

"Recombinant expression vector" refers to a replicable DNA construct used either to amplify or to express DNA which encodes IL-4R and which includes a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

"Recombinant microbial expression system" means a substantially homogeneous monoculture of suitable host microorganisms, for example, bacteria such as *E. coli* or yeast such as *S. cerevisiae*, which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit as a component of a resident plasmid. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined herein will express heterologous protein upon induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

### Proteins and Analogs

The present invention provides substantially homogeneous recombinant mammalian IL-4R polypeptides substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. The native murine and human IL-4 receptor molecules are recovered from cell lysates as glycoproteins having an apparent molecular weight by SDS-PAGE of about 130-145 kilodaltons (kDa). Mammalian IL-4R of the present invention includes, by way of example, primate, human, murine, canine, feline, bovine, ovine, equine and porcine IL-4R. Derivatives of IL-4R within the scope of the invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, an IL-4R protein may be in the form of acidic or basic salts, or in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to IL-4R amino acid side chains or at the N- or C-termini. Other derivatives of IL-4R within the scope of this invention include covalent or aggregative conjugates of IL-4R or its fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast  $\alpha$ -factor leader). IL-4R protein fusions can comprise peptides added to facilitate purification or identification of IL-4R (e.g., poly-His). The amino acid sequence of IL-4 receptor can also be linked to the peptide Asp-Tyr-Lys-Asp-Asp-Asp-Lys (DYKDDDDK) (Hopp et al., *Bio/Technology* 6:1204, 1988.) The latter sequence is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue immediately following the Asp-Lys pairing. Fusion proteins capped with this peptide may also be resistant to intracellular degradation in *E. coli*.

IL-4R derivatives may also be used as immunogens, reagents in receptor-based immunoassays, or as binding agents for affinity purification procedures of IL-4 or other binding ligands. IL-4R derivatives may also be obtained by cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. IL-4R proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, IL-4R may be used to selectively bind (for purposes of assay or purification) anti-IL-4R antibodies or IL-4.

The present invention also includes IL-4R with or without associated native-pattern glycosylation. IL-4R expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or significantly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of IL-4R DNAs in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of mammalian IL-4R having inactivated N-glycosylation sites can be

produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A<sub>1</sub>-Z, where A<sub>1</sub> is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A<sub>1</sub> and Z, or an amino acid other than Asn between Asn and A<sub>1</sub>.

IL-4R derivatives may also be obtained by mutations of IL-4R or its subunits. An IL-4R mutant, as referred to herein, is a polypeptide homologous to IL-4R but which has an amino acid sequence different from native IL-4R because of a deletion, insertion or substitution. Like most mammalian genes, mammalian IL-4 receptors are presumably encoded by multi-exon genes. Alternative mRNA constructs which can be attributed to different mRNA splicing events following transcription, and which share large regions of identity or similarity with the cDNAs claimed herein, are considered to be within the scope of the present invention.

Bioequivalent analogs of IL-4R proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those having physicochemical characteristics resembling those of the residue to be replaced. Similarly, when a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered.

Subunits of IL-4R may be constructed by deleting terminal or internal residues or sequences. Particularly preferred subunits include those in which the transmembrane region and intracellular domain of IL-4R are deleted or substituted with hydrophilic residues to facilitate secretion of the receptor into the cell culture medium. The resulting protein is a soluble IL-4R molecule which may retain its ability to bind IL-4. Particular examples of soluble IL-4R include polypeptides having substantial identity to the sequence of amino acid residues 1-208 in Figure 2A, and residues 1-207 in Figure 4A.

Mutations in nucleotide sequences constructed for expression of analog IL-4Rs must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed IL-4R mutants screened for the desired activity.

Not all mutations in the nucleotide sequence which encodes IL-4R will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*Bio Techniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Patent Nos. 4,518,584 and 4,737,462, which are incorporated by reference herein.

#### Expression of Recombinant IL-4R

The present invention provides recombinant expression vectors which include synthetic or cDNA-derived DNA fragments encoding mammalian IL-4R or bioequivalent analogs operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control

transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation, as described in detail below. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. DNA regions are operably linked when they are functionally  
 5 related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in  
 10 reading frame.

DNA sequences encoding mammalian IL-4 receptors which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA; however, premature termination of transcription may be desirable, for example, where it would result in mutants having advantageous C-terminal truncations, for example, deletion of a transmembrane region to yield a  
 15 soluble receptor not bound to the cell membrane. Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence; exemplary DNA embodiments are those corresponding to the nucleotide sequences shown in the Figures. Other embodiments include sequences capable of hybridizing to the sequences of the Figures under moderately stringent conditions (50° C, 2 X SSC) and other sequences hybridizing or degenerate to those described above, which encode  
 20 biologically active IL-4 receptor polypeptides.

Transformed host cells are cells which have been transformed or transfected with IL-4R vectors constructed using recombinant DNA techniques. Transformed host cells ordinarily express IL-4R, but host cells transformed for purposes of cloning or amplifying IL-4R DNA do not need to express IL-4R. Expressed  
 25 IL-4R will be deposited in the cell membrane or secreted into the culture supernatant, depending on the IL-4R DNA selected. Suitable host cells for expression of mammalian IL-4R include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce mammalian IL-4R using RNAs derived from the DNA constructs of the present invention. Appropriate  
 30 cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of IL-4Rs that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more  
 35 phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These  
 40 pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolívar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the  $\beta$ -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature*  
 50 281:544, 1979), the tryptophan (*trp*) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 38,778) and *tac* promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage  $\lambda$  *P<sub>L</sub>* promoter and c1857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the  $\lambda$  *P<sub>L</sub>* promoter include plasmid pHUB2, resident in *E. coli*  
 55 strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

Recombinant IL-4R proteins may also be expressed in yeast hosts, preferably from the *Saccharomyces* genus, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2 $\mu$  yeast plasmid or

an autonomously replicating sequence (ARS), promoter, DNA encoding IL-4R, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* *trp1* gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in *E. coli* (Amp<sup>r</sup> gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and  $\alpha$ -factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). The yeast  $\alpha$ -factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:933, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978, selecting for Trp<sup>r</sup> transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10  $\mu$ g/ml adenine and 20  $\mu$ g/ml uracil.

Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80  $\mu$ g/ml adenine and 80  $\mu$ g/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and either 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the viral origin of replication is included. Further, mammalian genomic IL-4R promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Additional details regarding the use of a mammalian high expression vectors to produce a recombinant mammalian IL-4 receptor are provided in Example 8 below. Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986).

A particularly preferred eukaryotic vector for expression of IL-4R DNA is disclosed below in Example 2. This vector, referred to as pCAV NOT, was derived from the mammalian high expression vector pDC201 and contains regulatory sequences from SV40, adenovirus-2, and human cytomegalovirus. pCAV NOT containing a human IL-7 receptor insert has been deposited with the American Type Culture Collection (ATCC) under deposit accession number 68014.

Purified mammalian IL-4 receptors or analogs are prepared by culturing suitable host vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts.

For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise an IL-4 or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify an IL-4R composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant mammalian IL-4R can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express mammalian IL-4R as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

Human IL-4R synthesized in recombinant culture is characterized by the presence of non-human cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover human IL-4R from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of IL-4R free of proteins which may be normally associated with IL-4R as it is found in nature in its species of origin, e.g. in cells, cell exudates or body fluids.

IL-4R compositions are prepared for administration by mixing IL-4R having the desired degree of purity with physiologically acceptable carriers. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the IL-4R with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients.

IL-4R compositions may be used to regulate the function of B cells. For example, soluble IL-4R (sIL-4R) inhibits the proliferation of B cell cultures induced by IL-4 in the presence of anti-Ig. sIL-4R also inhibits IL-4 induced IgG1 secretion by LPS-activated B cells as determined by isotype specific ELISA and inhibits IL-4 induced IgA expression on murine B cells as determined by EPICS analysis. sIL-4R also inhibits IL-4 induced IgE synthesis and may accordingly be used to treat IgE-induced immediate hypersensitivity reactions, such as allergic rhinitis (common hay fever), bronchial asthma, atopic dermatitis and gastrointestinal food allergy.

IL-4R compositions may also be used to regulate the function of T cells. For example, IL-4R inhibits IL-4 induced proliferation of T cell lines, such as the CTLL T cell line. sIL-4R also inhibits functional activity mediated by endogenously produced IL-4. For example, sIL-4R inhibits the generation of alloreactive cytolytic T lymphocytes (CTL) in secondary mixed leukocyte culture when present in culture concomitantly with a monoclonal antibody against IL-2, such as S4B6. Neutralizing agents for both IL-2 and IL-4 are used to inhibit endogenous IL-2 and IL-4, both of which regulate CTL generation and are produced in such cultures.

In therapeutic applications, a therapeutically effective quantity of an IL-4 receptor composition is administered to a mammal, preferably a human, in association with a pharmaceutical carrier or diluent.

The following examples are offered by way of illustration, and not by way of limitation.

## EXAMPLES

### Example 1

#### Binding assays for IL-4 receptor

A. *Radiolabeling of IL-4.* Recombinant murine and human IL-4 were expressed in yeast and purified to homogeneity as described by Park, et al., *Proc. Natl. Acad. Sci. USA* 84:5267(1987) and Park et al., *J. Exp. Med.* 166:476(1987), respectively. The purified protein was radiolabeled using a commercially available  
 20 enzymobead radioiodination reagent (BioRad). In this procedure 2.5  $\mu$ g rIL-4 in 50  $\mu$ l 0.2 M sodium phosphate, pH 7.2 are combined with 50  $\mu$ l enzymobead reagent, 2 MCi of sodium iodide in 20  $\mu$ l of 0.05 M sodium phosphate pH 7.0 and 10  $\mu$ l of 2.5% b-D-glucose. After 10 min at 25°C, sodium azide (10  $\mu$ l of 50 mM) and sodium metabisulfite (10  $\mu$ l of 5 mg/ml) were added and incubation continued for 5 min. at 25°C. The reaction mixture was fractionated by gel filtration on a 2 ml bed volume of Sephadex® G-25  
 25 (Sigma) equilibrated in Roswell Park Memorial Institute (RPMI) 1640 medium containing 2.5% (w/v) bovine serum albumin (BSA), 0.2% (w/v) sodium azide and 20 mM Hepes pH 7.4 (binding medium). The final pool of  $^{125}$ I-IL-4 was diluted to a working stock solution of  $2 \times 10^{-8}$  M in binding medium and stored for up to one month at 4°C without detectable loss of receptor binding activity. The specific activity is routinely in the range of  $1-2 \times 10^6$  cpm/mmol IL-4.

B. *Binding to Adherent Cells.* Binding assays done with cells grown in suspension culture (i.e.,  
 30 CTLL and CTLL-19.4) were performed by a phthalate oil separation method (Dower et al., *J. Immunol.* 132:751, 1984) essentially as described by Park et al., *J. Biol. Chem.* 261:4177, 1986 and Park et al., *supra*. Binding assays were also done on COS cells transfected with a mammalian expression vector containing cDNA encoding an IL-4 receptor molecule. For Scatchard analysis of binding to adherent cells,  
 35 COS cells were transfected with plasmid DNA by the method of Luthman et al., *Nucl. Acids. Res.* 11:1295, 1983, and McCutchan et al., *J. Natl. Cancer Inst.* 41:351, 1988. Eight hours following transfection, cells were trypsinized, and reseeded in six well plates (Costar, Cambridge, MA) at a density of  $1 \times 10^4$  COS-IL-4 receptor transfectants/well mixed with  $5 \times 10^5$  COS control transfected cells as carriers. Two days later monolayers were assayed for  $^{125}$ I-IL-4 binding at 4°C essentially by the method described by Park et al., *J.*  
 40 *Exp. Med.* 166:476, 1987. Nonspecific binding of  $^{125}$ I-IL-4 was measured in the presence of a 200-fold or greater molar excess of unlabeled IL-4. Sodium azide (0.2%) was included in all binding assays to inhibit internalization of  $^{125}$ I-IL-4 by cells at 37°C.

For analysis of inhibition of binding by soluble IL-4R, supernatants from COS cells transfected with recombinant IL-4R constructs were harvested three days after transfection. Serial two-fold dilutions of  
 45 conditioned media were pre-incubated with  $3 \times 10^{-10}$  M  $^{125}$ I-IL-4 (having a specific activity of about  $1 \times 10^6$  cpm/mmol) for one hour at 37°C prior to the addition of  $2 \times 10^6$  CTLL cells. Incubation was continued for 30 minutes at 37°C prior to separation of free and cell-bound murine  $^{125}$ I-IL-4.

C. *Solid Phase Binding Assays.* The ability of IL-4 receptor to be stably adsorbed to nitrocellulose from detergent extracts of CTLL 19.4 cells yet retain IL-4 binding activity provided a means of monitoring  
 50 purification. One ml aliquots of cell extracts (see Example 3), IL-4 affinity column fractions (see Example 4) or other samples are placed on dry BA85/21 nitrocellulose membranes (Schleicher and Schuell, Keene, NH) and allowed to dry. The membranes are incubated in tissue culture dishes for 30 minutes in Tris (0.05 M) buffered saline (0.15 M) pH 7.5 containing 3% w/v BSA to block nonspecific binding sites. The membrane is then covered with  $4 \times 10^{-11}$  M  $^{125}$ I-IL-4 in PBS + 3% BSA with or without a 200 fold molar excess of  
 55 unlabeled IL-4 and incubated for 2 hr at 4°C with shaking. At the end of this time, the membranes are washed 3 times in PBS, dried and placed on Kodak X-Omat™ AR film for 18 hr at -70°C.

## Example 2

5 Selection of CTLL cells with high IL-4 receptor expression by fluorescence activated cell sorting (FACS)

The preferred cell line for obtaining high IL-4 receptor selection is CTLL, a murine IL-2 dependent cytotoxic T cell line (ATCC TIB 214). To obtain higher levels of IL-4 receptor expression, CTLL cells (parent  
 10 cells) were sorted using fluorescence-activated cell sorting and fluorescein-conjugated recombinant murine IL-4 (rmIL-4) in which the extensive carbohydrate attached to rmIL-4 by the yeast host is used to advantage by coupling fluorescein hydrazide to periodate oxidized sugar moieties. The fluorescein-conjugated IL-4 was prepared by combining aliquots of hyperglycosylated rmIL-4 (300  $\mu$ g in 300  $\mu$ l of 0.1 M citrate-phosphate buffer, pH 5.5) with 30  $\mu$ l of 10 mM sodium m-periodate (Sigma), freshly prepared in 0.1 M citrate-phosphate,  
 15 phosphate, pH 5.5 and the mixture incubated at 4 °C for 30 minutes in the dark. The reaction was quenched with 30  $\mu$ l of 0.1 M glycerol and dialyzed for 18 hours at 4 °C against 0.1 M citrate-phosphate pH 5.5. Following dialysis, a 1:10 volume of 100 mM 5-(((2-(carbohydrazino)methyl)thio)acetyl)-aminofluorescein (Molecular Probes, Eugene OR) dissolved in DMSO was added to the sample and incubated at 25 °C for 30 minutes. The IL-4-fluorescein was then exhaustively dialyzed at 4 °C against PBS, pH 7.4 and protein  
 20 concentration determined by amino acid analysis. The final product was stored at 4 °C following the addition of 1% (w/v) BSA and sterile filtration.

In order to sort, CTLL cells ( $5 \times 10^6$ ) were incubated for 30 min at 37 °C in 150  $\mu$ l PBS + 1% BSA containing  $1 \times 10^{-9}$  M IL-4-fluorescein under sterile conditions. The mixture was then chilled to 4 °C, washed once in a large volume of PBS + 1% BSA and sorted using an EPICS® C flow cytometer (Coulter  
 25 Instruments). The cells providing the highest level fluorescence signal (top 1.0%) were collected in bulk and the population expanded in liquid cell culture. Alternatively, for single cell cloning, cells exhibiting a fluorescence signal in the top 1.0% were sorted into 96 well tissue culture microtiter plates at 1 cell per well.

Progress was monitored by doing binding assays with  $^{125}$ I-IL-4 following each round of FACS selection.  
 30 Unsorted CTLL cells (CTLL parent) typically exhibited 1000-2000 IL-4 receptors per cell. CTLL cells were subjected to 19 rounds of FACS selection. The final CTLL cells selected (CTLL-19) exhibited  $5 \times 10^5$  to  $1 \times 10^6$  IL-4 receptors per cell. At this point the CTLL-19 population was subjected to EPICS® C-assisted single cell cloning and individual clonal populations were expanded and tested for  $^{125}$ I-IL-4 binding. A single clone, designated CTLL-19.4, exhibited  $1 \times 10^6$  IL-4 receptors per cell and was selected for purification and  
 35 cloning studies. While the calculated apparent  $K_d$  values are similar for the two lines, CTLL-19.4 expresses approximately 400-fold more receptors on its surface than does the CTLL parent.

## Example 3

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Detergent extraction of CTLL cells

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CTLL 19.4 cells were maintained in RPMI 1640 containing 10% fetal bovine serum, 50 U/ml penicillin, 50  $\mu$ g/ml streptomycin and 10 ng/ml of recombinant human IL-2. Cells were grown to  $5 \times 10^5$  cells/ml in roller bottles, harvested by centrifugation, washed twice in serum free DMEM and sedimented at 2000 x g for 10 minutes to form a packed pellet (about  $2 \times 10^8$  cells/ml). To the pellet was added an equal volume of  
 50 PBS containing 1% Triton® X-100 and a cocktail of protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 10  $\mu$ M pepstatin, 10  $\mu$ M leupeptin, 2 mM o-phenanthroline and 2 mM EGTA). The cells were mixed with the extraction buffer by vigorous vortexing and the mixture incubated on ice for 20 minutes after which the mixture was centrifuged at 12,000 x g for 20 minutes at 8 °C to remove nuclei and other debris. The supernatant was either used immediately or stored at -70 °C until use.

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## Example 4

IL-4 receptor purification by IL-4 affinity chromatography

In order to obtain sufficient quantities of murine IL-4R to determine its N-terminal sequence or to further characterize human IL-4R, protein obtained from the detergent extraction of cells was further purified by affinity chromatography. Recombinant murine or human IL-4 was coupled to Affigel®-10 (BioRad) according to the manufacturer's suggestions. For example, to a solution of IL-4 (3.4 mg/ml in 0.4 ml of 0.1 M Hepes pH 7.4) was added 1.0 ml of washed Affigel®-10. The solution was rocked overnight at 4 °C and an aliquot of the supernatant tested for protein by a BioRad protein assay per the manufacturer's instructions using BSA as a standard. Greater than 95% of the protein had coupled to the gel, suggesting that the column had a final load of 1.3 mg IL-4 per ml gel. Glycine ethyl ester was added to a final concentration of 0.05 M to block any unreacted sites on the gel. The gel was washed extensively with PBS-1% Triton® followed by 0.1 M Glycine-HCl, pH 3.0. A 0.8 x 4.0 cm column was prepared with IL-4-coupled Affigel® prepared as described (4.0 ml bed volume) and washed with PBS containing 1% Triton® X-100 for purification of murine IL-4R. Alternatively, 50 µl aliquots of 20% suspension of IL-4-coupled Affigel® were incubated with <sup>35</sup>S-cysteine-methionine-labeled cell extracts for small-scale affinity purifications and gel electrophoresis.

Aliquots (25 ml) of detergent extracted IL-4 receptor bearing CTLL 19.4 cells were slowly applied to the murine IL-4 affinity column at 4 °C (flow rate of 3.0 ml/hr). The column was then washed sequentially with PBS containing 1% Triton® X-100, RIPA buffer (0.05 M Tris, 0.15 M NaCl, 1% NP-40, 1% deoxycholate and 0.1% SDS), PBS containing 0.1% Triton® X-100 and 10 mM ATP, and PBS with 1% Triton® X-100 to remove all contaminating material except the mIL-4R. The column was then eluted with pH 3.0 glycine HCl buffer containing 0.1% Triton® X-100 to remove the IL-4R and washed subsequently with PBS containing 0.1% Triton® X-100. One ml fractions were collected for the elution and 2 ml fractions collected during the wash. Immediately following elution, samples were neutralized with 80 µl of 1 M Hepes, pH 7.4. The presence of receptor in the fractions was detected by the solid phase binding assay as described above, using <sup>125</sup>I-labeled IL-4. Aliquots were removed from each fraction for analysis by SDS-PAGE and the remainder frozen at -70 °C until use. For SDS-PAGE, 40 µl of each column fraction was added to 40 µl of 2 X SDS sample buffer (0.125 M Tris HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol). The samples were placed in a boiling water bath for 3 minutes and 80 µl aliquots applied to sample wells of a 10% polyacrylamide gel which was set up and run according to the method of Laemmli (*Nature* 227:680, 1970). Following electrophoresis, gels were silver stained as previously described by Urdal et al. (*Proc. Natl. Acad. Sci. USA* 81: 6481, 1984).

Purification by the foregoing process permitted identification by silver staining of polyacrylamide gels of two mIL-4R protein bands averaging 45 - 55 kDa and 30 - 40 kDa that were present in fractions exhibiting IL-4 binding activity. Experiments in which the cell surface proteins of CTLL-19.4 cells were radiolabeled and <sup>125</sup>I-labeled receptor was purified by affinity chromatography suggested that these two proteins were expressed on the cell surface. The ratio of the lower to higher molecular weight bands increased upon storage of fractions at 4 °C, suggesting a precursor product relationship, possibly due to slow proteolytic degradation. The mIL-4 receptor protein purified by the foregoing process remains capable of binding IL-4, both in solution and when adsorbed to nitrocellulose.

Example 5Sequencing of IL-4 receptor protein

CTLL 19.4 mIL-4 receptor containing fractions from the mIL-4 affinity column purification were prepared for amino terminal protein sequence analysis by fractionating on an SDS-PAGE gel and then transferred to a PVDF membrane. Prior to running the protein fractions on polyacrylamide gels, it was first necessary to remove residual detergent from the affinity purification process. Fractions containing proteins bound to the mIL-4 affinity column from three preparations were thawed and concentrated individually in a speed vac under vacuum to a final volume of 1 ml. The concentrated fractions were then adjusted to pH 2 by the addition of 50% (v/v) TFA and injected onto a Brownlee RP-300 reversed-phase HPLC column (2.1 x 30 mm) equilibrated with 0.1% (v/v) TFA in H<sub>2</sub>O at a flow rate of 200 µl/min running on a Hewlett Packard Model 1090M HPLC. The column was washed with 0.1% TFA in H<sub>2</sub>O for 20 minutes post injection. The

HPLC column containing the bound protein was then developed with a gradient as follows:

Time	% Acetonitrile in 0.1% TFA
0	0
5	30
15	30
25	70
30	70
35	100
40	0

1 ml fractions were collected every five minutes and analyzed for the presence of protein by SDS PAGE followed by silver staining.

Each fraction from the HPLC run was evaporated to dryness in a speed vac and then resuspended in Laemmli reducing sample buffer, prepared as described by Laemmli, *U.K. Nature* 227:680, 1970. Samples were applied to a 5-20% gradient Laemmli SDS gel and run at 45 mA until the dye front reached the bottom of the gel. The gel was then transferred to PVDF paper and stained as described by Matsudaira, *J. Biol. Chem.* 262:10035, 1987. Staining bands were clearly identified in fractions from each of the three preparations at approximately 30,000 to 40,000 M<sub>r</sub>.

The bands from the previous PVDF blotting were excised and subjected to automated Edman degradation on an Applied Biosystems Model 477A Protein Sequencer essentially as described by March et al. (*Nature* 315:641, 1985), except that PTH amino acids were automatically injected and analyzed on line with an Applied Biosystems Model 120A HPLC using a gradient and detection system supplied by the manufacturer. The following amino terminal sequence was determined from the results of sequencing: NH<sub>2</sub>-Ile-Lys-Val-Leu-Gly-Glu-Pro-Thr-Cys-Asn-Phe-Ser-Asp-Tyr-Ile. The bands from the second preparation used for amino terminal sequencing were treated with CNBr using the *in situ* technique described by March et al. (*Nature* 315: 641, 1985) to cleave the protein after internal methionine residues. Sequencing of the resulting cleavage products yielded the following data, indicating that the CNBr cleaved the protein after two internal methionine residues:

Cycle	Residues Observed
1	Val, Ser
2	Gly, Leu
3	Ile, Val
4	Tyr, Ser
5	Arg, Tyr
6	Glu, Thr
7	Asp, Ala
8	Asn, Leu
9	Pro, Val
10	Ala
11	Glu, Val
12	Phe, Gly
13	Ile, Asn
14	Val, Gln
15	Tyr, Ile
16	Lys, Asn
17	Val, Thr
18	Thr, Gly

When compared with the protein sequences derived from clones 16 and 18 (see Figure 2), the sequences

matched as follows:

	1	5	10	15	18
Sequence 1:	(Met)-Val-Asn-Ile-Ser-Arg-Glu-Asp-Asn-Pro-Ala-Glu-Phe-Ile-Val-Tyr-Asn-Val-Thr				
Sequence 2:	(Met)-Ser-Gly-Val-Tyr-Tyr-Thr-Ala-Arg-Val-Arg-Val-Arg-Ser-Gln-Ile-Leu-Thr-Gly				

Identical matches were found for all positions of sequence 1 except Asn(2) and sequence 2, except Arg at positions 8, 10, and 12. Ser at position 13, and Leu at position 16. The above sequences correspond to amino acid residues 137-154 and 169-187 of Figure 2.

In addition, the amino terminal sequence matched a sequence derived from the clone with position 9 being defined as a Cys.

The above data support the conclusion that clones 16 and 18 are derived from the message for the IL-4 receptor.

### Example 6

#### Synthesis of hybrid-subtracted cDNA probe

In order to screen a library for clones encoding a murine IL-4 receptor, a highly enriched IL-4 receptor cDNA probe was obtained using a subtractive hybridization strategy. Polyadenylated (polyA<sup>+</sup>) mRNA was isolated from two similar cell lines, the parent cell line CTLL (which expresses approximately 2,000 receptors per cell) and the sorted cell line CTLL 19.4 (which expresses  $1 \times 10^6$  receptors per cell). The mRNA content of these two cell lines is expected to be identical except for the relative level of IL-4 receptor mRNA. A radiolabeled single-stranded cDNA preparation was then made from the mRNA of the sorted cell line CTLL 19.4 by reverse transcription of polyadenylated mRNA from CTLL 19.4 cells by a procedure similar to that described by Maniatis et al., *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982). Briefly, polyA<sup>+</sup> mRNA was purified as described by March et al. (*Nature* 315:641-647, 1985) and copied into cDNA by reverse transcriptase using oligo dT as a primer. To obtain a high level of <sup>32</sup>P-labeling of the cDNA, 100  $\mu$ Ci of <sup>32</sup>P-dCTP (s.a. = 3000 Ci/mmol) was used in a 50  $\mu$ l reaction with non-radioactive dCTP at 10  $\mu$ M. After reverse transcription at 42 °C for 2 hours, EDTA was added to 20 mM and the RNA was hydrolyzed by adding NaOH to 0.2 M and incubating the cDNA mixture at 68 °C for 20 minutes. The single-stranded cDNA was extracted with a phenol/chloroform (50/50) mixture previously equilibrated with 10 mM Tris-Cl, 1 mM EDTA. The aqueous phase was removed to a clean tube and made alkaline again by the addition of NaOH to 0.5 M. The cDNA was then size-fractionated by chromatography on a 6 ml Sephadex® G50 column in 30mM NaOH and 1 mM EDTA to remove small molecular weight contaminants.

The resulting size-fractionated cDNA generated from the sorted CTLL 19.4 cells was then hybridized with an excess of mRNA from the unsorted parental CTLL cells by ethanol-precipitating the cDNA from CTLL 19.4 cells with 30  $\mu$ g of polyA<sup>+</sup> mRNA isolated from unsorted CTLL cells, resuspending in 16  $\mu$ l of 0.25 M NaPO<sub>4</sub>, pH 6.8, 0.2% SDS, 2 mM EDTA and incubating for 20 hours at 68 °C. The cDNAs from the sorted CTLL 19.4 cells that are complementary to mRNAs from the unsorted CTLL cells form double stranded cDNA/mRNA hybrids, which can then be separated from the single stranded cDNA based on their different binding affinities on hydroxyapatite. The mixture was diluted with 30 volumes of 0.02 M NaPO<sub>4</sub>, pH 6.8, bound to hydroxyapatite at room temperature, and single-stranded cDNA was then eluted from the resin with 0.12 M NaPO<sub>4</sub>, pH 6.8, at 60 °C, as described by Sims et al., *Nature* 312:541, 1984. Phosphate buffer was then removed by centrifugation over 2 ml Sephadex® G50 spin columns in water. This hybrid subtraction procedure removes a majority of common sequences between CTLL 19.4 and unsorted CTLL cells, and leaves a single-stranded cDNA pool enriched for radiolabeled IL-4 receptor cDNA which can be used to probe a cDNA library (as described below).

## Example 7

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Synthesis of cDNA library and plaque screening

A cDNA library was constructed from polyadenylated mRNA isolated from CTLL 19.4 cells using standard techniques (Gubler, et al., *Gene* 25:263, 1983; Ausubel et al., eds., *Current Protocols in Molecular Biology*, Vol. 1, 1987). After reverse transcription using oligo dT as primer, the single-stranded CDNA was rendered double-stranded with DNA polymerase I, blunt-ended with T4 DNA polymerase, methylated with *EcoR* I methylase to protect *EcoR* I cleavage sites within the cDNA, and ligated to *EcoR* I linkers. The resulting constructs were digested with *EcoR* I to remove all but one copy of the linkers at each end of the cDNA, and ligated to an equimolar concentration of *EcoR* I cut and dephosphorylated  $\lambda$ ZAP<sup>®</sup> arms and the resulting ligation mix was packaged *in vitro* (Gigapack<sup>®</sup>) according to the manufacturer's instructions. Other suitable methods and reagents for generating cDNA libraries in  $\lambda$  phage vectors are described by Huynh et al., *DNA Cloning Techniques: A Practical Approach*, IRL Press, Oxford (1984); Meissner et al., *Proc. Natl. Acad. Sci. USA* 84:4171 (1987), and Ausubel et al., *supra*.  $\lambda$ ZAP<sup>®</sup> is a phage  $\lambda$  cloning vector similar to  $\lambda$ gt11 (U.S. Patent 4,788,135) containing plasmid sequences from pUC19 (Norrander et al., *Gene* 26:101, 1987), a polylinker site located in a lacZ gene fragment, and an f1 phage origin of replication permitting recovery of ssDNA when host bacteria are superinfected with f1 helper phage. DNA is excised in the form of a plasmid comprising the foregoing elements, designated Bluescript<sup>®</sup>. Gigapack<sup>®</sup> is a sonicated *E. coli* extract used to package  $\lambda$  phage DNA.  $\lambda$ ZAP<sup>®</sup>, Bluescript<sup>®</sup>, and Gigapack<sup>®</sup> are registered trademarks of Stratagene, San Diego, CA, USA.

The radiolabeled hybrid-subtracted cDNA from Example 6 was then used as a probe to screen the cDNA library. The amplified library was plated on BB4 cells at a density of 25,000 plaques on each of 20 150 mm plates and incubated overnight at 37°C. All manipulations of  $\lambda$ ZAP<sup>®</sup> and excision of the Bluescript<sup>®</sup> plasmid were as described by Short et al., (*Nucl. Acids Res.* 16:7583, 1988) and Stratagene product literature. Duplicate plaque lift filters were incubated with hybrid-subtracted cDNA probes from Example 6 in hybridization buffer containing 50% formamide, 5 X SSC, 5 X Denhardt's reagent and 10% dextran sulfate at 42°C for 48 hours as described by Wahl et al., *Proc. Natl. Acad. Sci. USA* 76:3683, 1979. Filters were then washed at 68°C in 0.2 x SSC. Sixteen positive plaques were purified for further analysis.

Bluescript<sup>®</sup> plasmids containing the cDNA inserts were excised from the phage as described by the manufacturer and transformed into *E. coli*. Plasmid DNA was isolated from individual colonies, digested with *EcoR* I to release the cDNA inserts and electrophoresed on standard 1% agarose gels. Four duplicate gels were blotted onto nylon filters to produce identical Southern blots for analysis with various probes which were (1) radiolabeled cDNA from unsorted CTLL cells, (2) radiolabeled cDNA from CTLL 19.4 sorted cells, (3) hybrid subtracted cDNA from CTLL 19.4 sorted cells, and (4) hybrid subtracted cDNA from CTLL 19.4 sorted cells after a second round of hybridization to poly A<sup>+</sup> mRNA from an IL-4 receptor negative mouse cell line (LBRM 33 1A5B6). These probes were increasingly enriched for cDNA copies of mRNA specific for the sorted cell line CTLL 19.4. Of the 16 positive plaques isolated from the library, four clones (11A, 14, 16 and 18) showed a parallel increase in signal strength with enrichment of the probe.

Restriction mapping (shown in Figure 1) and DNA sequencing of the isolated CTLL clones indicated the existence of at least two distinct mRNA populations. Both mRNA types have homologous open reading frames over most of the coding region yet diverge at the 3' end, thus encoding homologous proteins with different COOH-terminal sequences. DNA sequence from inside the open reading frames of both clones code for protein sequence that is identical to protein sequence derived from sequencing of the purified IL-4 receptor described in more detail in Example 5. Clone 16 and clone 18 were used as the prototypes for these two distinct message types. Clone 16 contains an open reading frame that encodes a 258-amino acid polypeptide which includes amino acids -25 to 233 of Figure 2A. Clone 18 encodes a 230-amino acid polypeptide, the N-terminal 224 amino acids of which are identical to the N-terminus of clone 16 but diverge at the 3' end with nucleotides CCAAGTAATGAAAATCTG which encode the C-terminal 6 amino acids, Pro-Ser-Asn-Glu-Asn-Leu, followed by a termination codon TGA. Both clones were expressed in a mammalian expression system, as described in Example 8.

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## Example 8

Expression of IL-4R in mammalian cells

A. *Expression in COS-7 Cells.* A eukaryotic expression vector pCAV NOT, shown in Figure 3, was derived from the mammalian high expression vector pDC201, described by Sims et al., *Science* 241:585, 1988). pDC201 is a derivative of pMLSV, previously described by Cosman et al., *Nature* 312:768, 1984. pCAV NOT is designed to express cDNA sequences inserted at its multiple cloning site (MCS) when transfected into mammalian cells and includes the following components: SV40 (hatched box) contains SV40 sequences from coordinates 5171-270 including the origin of replication, enhancer sequences and early and late promoters. The fragment is oriented so that the direction of transcription from the early promoter is as shown by the arrow. CMV contains the promoter and enhancer regions from human cytomegalovirus (nucleotides -671 to +7 from the sequence published by Boshart et al., *Cell* 41:521-530, 1985). The tripartite leader (stippled box) contains the first exon and part of the intron between the first and second exons of the adenovirus-2 tripartite leader, the second exon and part of the third exon of the tripartite leader and a multiple cloning site (MCS) containing sites for *Xho* I, *Kpn* I, *Sma* I, *Not* I and *Bgl* II. pA (hatched box) contains SV40 sequences from 4127-4100 and 2770-2533 that include the polyadenylation and termination signals for early transcription. Clockwise from pA are adenovirus-2 sequences 10532-11156 containing the VAI and VAI1 genes (designated by a black bar), followed by pBR322 sequences (solid line) from 4383-2486 and 1094-375 containing the ampicillin resistance gene and origin of replication. The resulting expression vector was designated pCAV NOT.

Inserts in clone 16 and clone 18 were both released from Bluescript® plasmid by digestion with *Asp* 718 and *Not* I. The 3.5 kb insert from clone 16 was then ligated directly into the expression vector pCAV NOT also cut at the *Asp* 718 and *Not* I sites in the polylinker region. The insert from clone 18 was blunt-ended with T4 polymerase followed by ligation into the vector pCAV NOT cut with *Sma* I and dephosphorylated.

Plasmid DNA from both IL-4 receptor expression plasmids were used to transfect a sub-confluent layer of monkey COS-7 cells using DEAE-dextran followed by chloroquine treatment, as described by Luthman et al. (*Nucl. Acids Res.* 11:1295, 1983) and McCutchan et al. (*J. Natl. Cancer Inst.* 41:351, 1968). The cells were then grown in culture for three days to permit transient expression of the inserted sequences. After three days, cell culture supernatants and the cell monolayers were assayed (as described in Example 1) and IL-4 binding was confirmed.

B. *Expression in CHO Cells.* IL-4R was also expressed in the mammalian CHO cell line by first ligating an *Asp*718/*Not*I restriction fragment of clone 18 into the pCAV NOT vector as described in Example 8. The pCAV NOT vector containing the insert from clone 18 was then co-transfected using a standard calcium phosphate method into CHO cells with the dihydrofolate reductase (DHFR) cDNA selectable marker under the control of the SV40 early promoter. The DHFR sequence enables methotrexate selection for mammalian cells harboring the plasmid. DHFR sequence amplification events in such cells were selected using elevated methotrexate concentrations. In this way, the contiguous DNA sequences are also amplified and thus enhanced expression is achieved. Mass cell cultures of the transfectants secreted active soluble IL-4R at approximately 100 ng/ml.

C. *Expression in HeLa Cells.* IL-4R was expressed in the human HeLa-EBNA cell line 653-6, which constitutively expresses EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. The expression vector used was pHAV-EO-NEO, described by Dower et al., *J. Immunol.* 142:4314, 1989), a derivative of pDC201, which contains the EBV origin of replication and allows high level expression in the 653-6 cell line. pHAV-EO-NEO is derived from pDC201 by replacing the adenovirus major late promoter with synthetic sequences from HIV-1 extending from -148 to +78 relative to the cap site of the viral mRNA, and including the HIV-1 *tat* gene under the control of the SV-40 early promoter. It also contains a *Bgl* II-*Sma* I fragment containing the neomycin resistance gene of pSV2NEO (Southern & Berg, *J. Mol. Appl. Genet.* 1:332, 1982) inserted into the *Bgl* II and *Hpa* I sites and subcloning downstream of the *Sal* I cloning site. The resulting vector permits selection of transfected cells for neomycin resistance.

A 760 bp IL-4R fragment was released from the Bluescript® plasmid by digesting with *Eco*N I and *Sst* I restriction enzymes. This fragment of clone 18 corresponds to nucleotides 1-672 of Figure 2A, with the addition of a 5' terminal nucleotide sequence of GTGCAGGCACCTTTTGTGTCCCCA, a TGA stop codon which follows nucleotide 672 of Figure 2A, and a 3' terminal nucleotide sequence of CTGAGTGACCTTGGGGGCTGCGGTGGTGAGGAGAGCT. This fragment was then blunt-ended using T4 polymerase and subcloned into the *Sal* I site of pHAV-EO-NEO. The resulting plasmid was then transfected into the 653-6 cell line by a modified polybrene transfection method as described by Dower et al. (*J. Immunol.* 142:4314, 1989) with the exception that the cells were trypsinized at 2 days post-transfection and split at a ratio of 1:8

into media containing G418 (Gibco Co.) at a concentration of 1 mg/ml. Culture media were changed twice weekly until neomycin-resistant colonies were established. Colonies were then either picked individually using cloning rings, or pooled together, to generate several different cell lines. These cell lines were maintained under drug selection at a G418 concentration of 250 µg/ml. When the cells reached confluency supernatants were taken and tested in the inhibition assay of Example 1B. Cell lines produced from 100 ng/ml to 600 ng/ml of soluble IL-4R protein.

### Example 9

#### Expression of IL-4R in yeast cells

For expression of mIL-4R, a yeast expression vector derived from pIXY120 was constructed as follows. pIXY120 is identical to pYαHuGM (ATCC 53157), except that it contains no cDNA insert and includes a polylinker multiple cloning site with an *Nco* I site. This vector includes DNA sequences from the following sources: (1) a large *Sph* I (nucleotide 562) to *Eco*R I (nucleotide 4361) fragment excised from plasmid pBR322 (ATCC 37017), including the origin of replication and the ampicillin resistance marker for selection in *E. coli*; (2) *S. cerevisiae* DNA including the TRP-1 marker, 2µ origin of replication, ADH2 promoter; and (3) DNA encoding an 85 amino acid signal peptide derived from the gene encoding the secreted peptide α-factor (See Kurjan et al., U.S. Patent 4,546,082). An *Asp* 718 restriction site was introduced at position 237 in the α-factor signal peptide to facilitate fusion to heterologous genes. This was achieved by changing the thymidine residue at nucleotide 241 to a cytosine residue by oligonucleotide-directed *in vitro* mutagenesis as described by Craik, *BioTechniques*, January 1985, pp.12-19. A synthetic oligonucleotide containing multiple cloning sites and having the following sequence was inserted from the *Asp*718 site at amino acid 79 near the 3' end of the α-factor signal peptide to a *Spe*I site in the 2µ sequence:

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Asp718                               StuI   NcoI       BamHI SmaI       SpeI
GTACCTTTGGATAAAAGAGACTACAAGGACGACGATGACAAGAGGGCTCCATGGATCCCCCGGGACA
GAAACCTATTTTCTCTGATGTTCTGCTACTGTTCTCCGGAGGTACCTAGGGGGCCCTGTGATC
                                     |-----Polylinker-----|

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pBC120 also varies from pYαHuGM by the presence of a 514 bp DNA fragment derived from the single-stranded phage f1 containing the origin of replication and intergenic region, which has been inserted at the *Nru* I site in the pBR322 sequence. The presence of an f1 origin of replication permits generation of single-stranded DNA copies of the vector when transformed into appropriate strains of *E. coli* and superinfected with bacteriophage f1, which facilitates DNA sequencing of the vector and provides a basis for *in vitro* mutagenesis. To insert a cDNA, pIXY120 is digested with *Asp* 718 which cleaves near the 3' end of the α-factor leader peptide (nucleotide 237) and, for example, *Bam*HI which cleaves in the polylinker. The large vector fragment is then purified and ligated to a DNA fragment encoding the protein to be expressed.

To create a secretion vector for expressing mIL-4R, a cDNA fragment encoding mIL-4R was excised from the Bluescript® plasmid of Example 8 by digestion with *Ppu*m I and *Bgl* II to release an 831 bp fragment from the *Ppu*m I site (see FIGURE ) to an *Bgl* II site located 3' to the open reading frame containing the mIL-4R sequence minus the first two 5' codons encoding Ile and Lys. pIXY120 was digested with *Asp* 718 near the 3' end of the α-factor leader and *Bam*HI. The vector fragment was ligated to the *Ppu*m I/*Bgl* II mIL-4R cDNA fragment and the following fragment created by annealing a pair of synthetic oligonucleotides to recreate the last 6 amino acids of the α-factor leader and the first two amino acids of mature mIL-4R.

$\alpha$ -factor processing  $\rightarrow$ |  
 GTA CCT CTA GAT AAA AGA ATC AAG  
 GA GAT CTA TTT TCT TAG TTC CAG  
 Val Pro Leu Asp Lys Arg Ile Lys  
 ← mL-4R

The oligonucleotide also included a change from the nucleotide sequence TGG ATA to CTA GAT which introduces a *Xba*I restriction site, without altering the encoded amino acid sequence.

The foregoing expression vector was then purified and employed to transform a diploid yeast strain of *S. cerevisiae* (XV2181) by standard techniques, such as those disclosed in EPA 165,654, selecting for tryptophan prototrophs. The resulting transformants were cultured for expression of a secreted mL-4R protein. Cultures to be assayed for biological activity were grown in 20-50 ml of YPD medium (1% yeast extract, 2% peptone, 1% glucose) at 37°C to a cell density of  $1-5 \times 10^8$  cells/ml. To separate cells from medium, cells were removed by centrifugation and the medium filtered through a 0.45  $\mu$  cellulose acetate filter prior to assay. Supernatants produced by the transformed yeast strain, or crude extracts prepared from disrupted yeast cells transformed the plasmid, were assayed to verify expression of a biologically active protein.

### Example 10

#### Isolation of full-length and truncated forms of murine IL-4 receptor cDNAs from unsorted 7B9 cells

Polyadenylated RNA was isolated from 7B9 cells, an antigen-dependent helper T cell clone derived from C57BL/6 mice, and used to construct a cDNA library in  $\lambda$ ZAP (Stratagene, San Diego), as described in example 7. The  $\lambda$ ZAP library was amplified once and a total of 300,000 plaques were screened as described in Example 7, with the exception that the probe was a randomly primed  $^{32}$ P-labeled 700 bp *Eco*R I fragment isolated from CTLL 19.4 clone 18. Thirteen clones were isolated and characterized by restriction analysis.

Nucleic acid sequence analysis of clone 7B9-2 revealed that it contains a polyadenylated tail, a putative polyadenylation signal, and an open reading frame of 810 amino acids (shown in Fig. 2), the first 258 of which are identical to those encoded by CTLL 19.4 clone 18, including the 25 amino acid putative signal peptide sequence. The 7B9-2 cDNA was subcloned into the eukaryotic expression vector, pCAV/NOT, and the resulting plasmid was transfected into COS-7 cells as described in Example 8. COS-7 transfectants were analyzed as set forth in Example 12.

A second cDNA form, similar to clone 18 in the CTLL 19.4 library, was isolated from the 7B9 library and subjected to sequence analysis. This cDNA, clone 7B9-4, is 376 bp shorter than clone 7B9-2 at the 5' end, and lacks the first 47 amino acids encoded by 7B9-2, but encodes the remaining N-terminal amino acids 23-199 (in Fig. 2). At position 200, clone 7B9-4 (like clone 18 from CTLL 19.4) has a 114 bp insert which changes the amino acid sequence to Pro Ser Asn Glu Asn Leu followed by a termination codon. The 114 bp inserts, found in both clone 7B9-4 and CTLL 19.4 clone 18 are identical in nucleic acid sequence. The fact that this cDNA form, which produces a secreted form of the IL-4 receptor when expressed in COS-7 cells, was isolated from these two different cell lines indicates that it is neither a cloning artifact nor a mutant form peculiar to the sorted CTLL cells.

### Example 11

#### Isolation of human IL-4 receptor cDNAs from PBL and T22 libraries by cross-species hybridization

Polyadenylated RNA was isolated from pooled human peripheral blood lymphocytes (PBL) that were

obtained by standard Ficoll purification and were cultured in IL-2 for six days followed by stimulation with PMA and Con-A for eight hours. An oligo dT primed cDNA library was constructed in  $\lambda$ gt10 using techniques described in example 7. A probe was produced by synthesizing an unlabeled RNA transcript of the 7B9-4 cDNA insert using T7 RNA polymerase, followed by  $^{32}$ P-labeled cDNA synthesis with reverse transcriptase using random primers (Boehringer-Mannheim). This murine single-stranded cDNA probe was used to screen 50,000 plaques from the human cDNA library in 50% formamide-0.4 M NaCl at 42°C, followed by washing in 2 X SSC at 55°C. Three positive plaques were purified, and the *EcoR* I inserts subcloned into the Bluescript<sup>®</sup> plasmid vector. Nucleic acid sequencing of a portion of clone PBL-1, a 3.4 kb cDNA, indicated the clone was approximately 67% homologous to the corresponding sequence of the murine IL-4 receptor. However, an insert of 68 bp, containing a termination codon and bearing no homology to the mouse IL-4 receptor clones, was found 45 amino acids downstream of the predicted N-terminus of the mature protein, suggesting that clone PBL-1 encodes a non-functional truncated form of the receptor. Nine additional human PBL clones were obtained by screening the same library (under stringent conditions) with a  $^{32}$ P-labeled random-primed probe made from the clone PBL-1 (the 3.4 kb *EcoR* I cDNA insert). Two of these clones, PBL-11 and PBL-5, span the 5' region that contains the 68 bp insert in PBL-1, but lack the 68 bp insert and do not extend fully 3', as evidenced by their size, thus precluding functional analysis by mammalian expression. In order to obtain a construct expressible in COS-7 cells, the 5' *Not* I-*Hinc* II fragment of clones PBL-11 and PBL-5 were separately ligated to the 3' *Hinc* II-*Bam*HI end of clone PBL-1, and subcloned into the pCAV NOT expression vector cut with *Not* I and *Bgl* II described in Example 8. These chimeric human IL-4R cDNAs containing PBL-11/PBL-1 and PBL-5/PBL-1 DNA sequences have been termed clones A5 and B4, respectively, as further described in Example 12. These constructs were transfected into COS-7 cells, and assayed for IL-4 binding in a plate binding assay substantially as described in Sims et al. (*Science* 241:585, 1988). Both composite constructs encoded protein which exhibited IL-4 binding activity. The nucleotide sequence and predicted amino acid sequence of the composite A5 construct correspond to the sequence information set forth in Figures 4A-4C, with the exception that a GTC codon encodes the amino acid Val at position 50, instead of Ile. No other clones that were sequenced contained this change. The consensus codon from clones PBL-1, PBL-5 and T22-8, however, is ATC and encodes Ile<sup>50</sup>, as set forth in Figure 4A. The nucleotide and predicted amino acid sequence of the composite B4 construct also shows that the 25 amino acid leader sequence of PBL-11 is replaced with the sequence Met-Gln-Lys-Asp-Ala-Arg-Arg-Glu-Gly-Asn.

Constructs expressing a soluble form of the human IL-4 receptor were made by excising a 5'-terminal 0.8 kb *Sma* I-*Dra* III fragment from PBL-5 and the corresponding 0.8 kb *Asp*718-*Dra* III fragment from PBL-11, of which the *Dra* III overhangs were blunt-ended with T4 polymerase. The PBL-5 and PBL-11 fragments were separately subcloned into CAV/NOT cut with *Sma* I or *Asp* 718 plus *Sma* I, respectively; these are called soluble hIL-4R-5 and soluble hIL-4R-11, respectively. In both constructs the final IL-4 receptor amino acid Thr<sup>194</sup> codon is followed by the vector-encoding amino acids GlyGlnArgProLeuGlnIleTyrAlaIle before terminating.

A second library made from a CD4<sup>+</sup>/CD8<sup>-</sup> human T cell clone, T22, (Acres et al., *J.Immunol.* 138:2132, 1987) was screened (using duplicate filters) with two different probes synthesized as described above. The first probe was obtained from a 220 bp *Pvu* II fragment from the 5' end of clone PBL-1 and the second probe was obtained from a 300 bp *Pvu* II-*EcoR* I fragment from the 3' end of the clone PBL-1. Five additional cDNA clones were identified using these two probes. Two of these clones span the 5' region containing the 68 bp insert, but neither contain the insert. The third of these clones T22-8, was approximately 3.6 kb in size and contained in an open reading frame of 825 amino acids, including a 25 amino acid leader sequence, a 207 amino acid mature external domain, a 24 amino acid transmembrane region and a 569 amino acid cytoplasmic domain. The sequence of clone T22-8 is set forth in Figures 4A-4C. Figures 5A-5B compare the predicted human IL-4R amino acid sequence with the predicted murine IL-4R sequence and show approximately 53% sequence identity between the two proteins.

A third soluble human IL-4 receptor construct was made as follows. cDNA clone T22-8 was cleaved at the *Dra*III site in the Thr<sup>194</sup> codon, and repaired with synthetic oligonucleotides to regenerate Thr<sup>194</sup> and Lys<sup>195</sup> codons, followed by a termination codon, and a *Not*I restriction site. A 0.68 kb *Sty*I-*Not*I restriction fragment of this clone was then blunt-ended at the *Sty*I site and subcloned into a *Sma*I-*Not*I digested pCAV/NOT vector. This cDNA expression vector was designated hIL-4R-8.

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#### Example 12

Analysis and purification of IL-4 receptor in COS transfectants

Equilibrium binding studies were conducted for COS cells transfected with murine IL-4 receptor clones 18 and 18 from the CTLL 19.4 library. In all cases analysis of the data in the Scatchard coordinate system (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660-672, 1949) yielded a straight line, indicating a single class of high-affinity receptors for murine IL-4. For COS pCAV-16 cells the calculated apparent  $K_d$  was  $3.6 \times 10^3 \text{ M}^{-1}$  with  $5.9 \times 10^5$  specific binding sites per cell. A similar apparent  $K_d$  was calculated for COS pCAV-18 cells at  $1.5 \times 10^3 \text{ M}^{-1}$  but receptor number expressed at the cell surface was  $4.2 \times 10^4$ . Equilibrium binding studies performed on COS cells transfected with IL-4R DNA clones isolated from the 7B9 cell library also showed high affinity binding of the receptor to IL-4. Specifically, studies using COS cells transfected with pCAV-7B9-2 demonstrated that the full length murine IL-4 receptor bound  $^{125}\text{I}$ -IL-4 with an apparent  $K_d$  of about  $1.4 \times 10^3 \text{ M}^{-1}$  with  $4.5 \times 10^4$  specific binding sites per cell. The apparent  $K_d$  of CAV-7B9-4 IL-4R was calculated to be about  $1.7 \times 10^3 \text{ M}^{-1}$ . Although absolute values for  $K_d$  and binding sites per cell varied between transfections, the binding affinities were generally similar ( $1 \times 10^3 - 1 \times 10^3 \text{ M}^{-1}$ ) and matched well with previously published affinity constants for IL-4 binding.

Inhibition of  $^{125}\text{I}$ -mIL-4 binding to CTLL cells by conditioned media from COS cells transfected with plasmid pCAV, pCAV-18, or pCAV-7B9-4 was used to determine if these cDNAs encoded functional soluble receptor molecules. Approximately 1.5  $\mu\text{l}$  of COS pCAV-18 conditioned media in a final assay volume of 150  $\mu\text{l}$  gives approximately 50% inhibition of  $^{125}\text{I}$ -IL-4 binding to the IL-4 receptor on CTLL cells.  $^{125}\text{I}$ -IL-4 receptor competing activity is not detected in control pCAV transfected COS supernatants. From quantitative analysis of the dilution of pCAV-18 supernatant required to inhibit  $^{125}\text{I}$ -IL-4 binding by 50%, it is estimated that approximately 60-100 ng/ml of soluble IL-4 receptor has been secreted by COS cells when harvested three days after transfection. Similar results were obtained utilizing supernatants from COS cells transfected with pCAV-7B9-4.

Conditioned medium from COS cells transfected with pCAV-18 or pCAV-7B9-4 (see Example 8) and grown in DMEM containing 3% FBS was harvested three days after transfection. Supernatants were centrifuged at 3,000 cpm for 10 minutes, and frozen until needed. Two hundred ml of conditioned media was loaded onto a column containing 4 ml of mull-4 Affigel prepared as described above. The column was washed extensively with PBS and IL-4 receptor eluted with 0.1 M glycine, 0.15 M NaCl pH 3.0. Immediately following elution, samples were neutralized with 80  $\mu\text{l}$  of 1 M Hepes pH 7.4. Samples were tested for their ability to inhibit binding of  $^{125}\text{I}$ -mull-4 to CTLL cells as set forth in Example 1B. Additionally samples were tested for purity by analysis on SDS-PAGE and silver staining as previously described. Alternative methods for testing functional soluble receptor activity or IL-4 binding inhibition include solid-phase binding assays, as described in Example 1C, or other similar cell free assays which may utilize either radio iodinated or colorimetrically developed IL-4 binding, such as RIA or ELISA. The protein analyzed by SDS-PAGE under reducing conditions has a molecular weight of approximately 37,500, and appears approximately 90% pure by silver stain analysis of gels.

Purified recombinant soluble murine IL-4 receptor protein may also be tested for its ability to inhibit IL-4 induced  $^3\text{H}$ -thymidine incorporation in CTLL cells. Pursuant to such methods, soluble IL-4 receptor has been found to block IL-4 stimulated proliferation, but does not affect IL-2 driven mitogenic response.

Molecular weight estimates were performed on mIL-4 receptor clones transfected into COS cells. Utilizing M2 monoclonal antibody prepared against murine CTLL 19.4 cells (see Example 13), IL-4 receptor is immunoprecipitated from COS cells transfected with CAV-18, CAV-7B9-2 and CAV-7B9-4 and labeled with  $^{35}\text{S}$ -cysteine and  $^{35}\text{S}$ -methionine. Cell associated receptor from CAV-7B9-4 shows molecular weight heterogeneity ranging from 32-39 kDa. Secreted CAV-7B9-4 receptor has molecular weight between 36 and 41 kDa. Cell associated receptor from CAV-16 transfected COS cells is about 40-41 kDa. This is significantly smaller than molecular weight estimations from crosslinking studies described by Park et al., *J. Exp. Med.* 166:476, 1987; *J. Cell. Biol., Suppl.* 12A, 1988. Immunoprecipitation of COS CAV-7B9-2 cell-associated receptor showed a molecular weight of 130-140 kDa, similar to the estimates of Park et al., *J. Cell. Biol., Suppl.* 12A, 1988, estimated to be the full length IL-4 receptor. Similar molecular weight estimates of cell-associated CAV-16 and CAV-7B9-2 IL-4 receptor have also been made based on cross-linking  $^{125}\text{I}$ -IL-4 to COS cells transfected with these cDNAs. Heterogeneity of molecular weight of the individual clones can be partially attributed to glycosylation. This data, together with DNA sequence analysis, suggests that the 7B9-2 cDNA encodes the full length cell-surface IL-4 receptor, whereas both 7B9-4 and clone 18 represent soluble forms of murine IL-4 receptor.

Receptor characterization studies were also done on COS cells transfected with hIL-4R containing expression plasmids. The two chimeric human IL-4R molecules A5 and B4 (defined in Example 11) were

transfected into COS cells and equilibrium binding studies undertaken. The COS monkey cell itself has receptors capable of binding hIL-4; therefore the binding calculations performed on COS cells transfected with and overexpressing hIL-4R cDNAs represent background binding from endogenous monkey IL-4R molecules subtracted from the total binding. COS cells transfected with hIL-4R A5 had  $5.3 \times 10^4$  hIL-4 binding sites with a calculated  $K_d$  of  $3.48 \times 10^9 \text{ M}^{-1}$ . Similarly, the hIL-4R B4 expressed in COS cells bound  $^{125}\text{I}$ -hIL-4 with an affinity of  $3.94 \times 10^9 \text{ M}^{-1}$  exhibiting  $3.2 \times 10^4$  receptors per cell.

Molecular weight estimates of human IL-4R expressed in COS cells were also performed. COS cells transfected with clones A5 or B4 in pCAV NOT were labeled with  $^{35}\text{S}$ -cysteine, methionine and lysed. Human IL-4R was affinity purified from the resulting lysates with hIL-4-coupled Affigel® (as described in Example 4). The hIL-4R A5 and B4 eluted from this affinity support migrated at about 140,000 daltons on SDS-PAGE, agreeing well with previous estimates of hIL-4R molecular weight by cross-linking (Park et al., *J. Exp. Med.* 166:476,1987), as well as with estimates of full-length mL-4R presented here.

Because no soluble human IL-4R cDNA has thus far been found occurring naturally, as was the case for the murine receptor (clones 18 and 7B9-4), a truncated form was constructed as described in Example 11. Following expression in COS cells, supernatants were harvested three days after transfection with soluble hIL-4R-11 and soluble hIL-4R-5 and tested for inhibition of  $^{125}\text{I}$ -hIL-4 binding to the human B cell line Raji. Supernatants from two of the soluble hIL-4R-11 and one of the soluble hIL-4R-5 transfected plates contained 29-149 ng/ml of IL-4R competing activity into the medium. In addition, the truncated protein could be detected in  $^{35}\text{S}$ -methionine-cysteine-labeled COS cell transfectants by affinity purification on hIL-4-coupled Affigel® as approximately a 44 kDa protein by SDS-PAGE. Supernatants COS cells transfected with hIL-4R-8 (encoding soluble truncated IL-4R) when concentrated 25-fold, inhibited human IL-4 binding to Raji cells, and contained approximately 16 ng/ml of competing activity.

## Example 13

### Preparation of monoclonal antibodies to IL-4R

Preparations of purified recombinant IL-4 receptor, for example, human or murine IL-4 receptor, transfected COS cells expressing high levels of IL-4 receptor or CTLL 19.4 cells are employed to generate monoclonal antibodies against IL-4 receptor using conventional techniques, such as those disclosed in U.S. Patent 4,411,993. Such antibodies are likely to be useful in interfering with IL-4 binding to IL-4 receptors, for example, in ameliorating toxic or other undesired effects of IL-4.

To immunize rats, IL-4 receptor bearing CTLL 19.4 cells were used as immunogen emulsified in complete Freund's adjuvant and injected in amounts ranging from 10-100  $\mu\text{l}$  subcutaneously into Lewis rats. Three weeks later, the immunized animals were boosted with additional immunogen emulsified in incomplete Freund's adjuvant and boosted every three weeks thereafter. Serum sample are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay, ELISA (enzyme-linked immunosorbent assay), or inhibition of binding of  $^{125}\text{I}$ -IL-4 to extracts of CTLL cells (as described in Example 1). Other assay procedures are also suitable. Following detection of an appropriate antibody titer, positive animals were given a final intravenous injection of antigen in saline. Three to four days later, the animals were sacrificed, splenocytes harvested, and fused to the murine myeloma cell line AG8653. Hybridoma cell lines generated by this procedure were plated in multiple microtiter plates in a HAT selective medium (hypoxanthine, aminopterin, and thymidine) to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

Hybridoma clones thus generated were screened for reactivity with IL-4 receptor. Initial screening of hybridoma supernatants utilized an antibody capture and binding of partially purified  $^{125}\text{I}$ -mIL-4 receptor. Two of over 400 hybridomas screened were positive by this method. These two monoclonal antibodies, M1 and M2, were tested by a modified antibody capture to detect blocking antibody. Only M1 was able to inhibit  $^{125}\text{I}$ -mIL-4 binding to intact CTLL cells. Both antibodies are capable of immunoprecipitating native mL-4R protein from CTLL cells or COS-7 cells transfected with IL-4R clones labelled with  $^{35}\text{S}$ -cysteine/methionine. M1 and M2 were then injected into the peritoneal cavities of nude mice to produce ascites containing high concentrations ( $>1 \text{ mg/ml}$ ) of anti-IL-4R monoclonal antibody. The resulting monoclonal antibody was purified by ammonium sulfate precipitation followed by gel exclusion chromatography, and/or affinity chromatography based on binding of antibody to Protein G.

Example 145      Use of soluble IL-4R to suppress immune response *in vivo*

Experiments were conducted to determine the effect of soluble IL-4R on allogeneic host versus graft (HVG) response *in vivo* using a popliteal lymph node assay. In this model mice are injected in the footpad  
 10 with irradiated, allogeneic spleen cells. Irradiated, syngeneic cells are then injected into the contralateral pad. An alloreactive response occurs in the pad receiving the allogeneic cells, the extent of which can be measured by the relative increase in size and weight of the popliteal lymph node draining the site of antigen deposition.

On day 0 three BALB.C mice were injected in the footpad with irradiated, allogeneic spleen cells from  
 15 c57BL/6 mice and in the contralateral footpad with irradiated, syngeneic spleen cells. On days -1.0 and +1 three mice were injected (intravenously on days -1 and 0, and subcutaneously on day +1) with 100 ng of purified soluble IL-4R (sIL-4R) in phosphate buffered saline, three mice were injected intravenously with 1µg of sIL-4R, three mice were injected with 2µg of sIL-4R and three mice were injected with MSA (control). The mean difference in weight of the lymph nodes from the sites of allogeneic and syngeneic  
 20 spleen cells was approximately 2.5 mg for the mice treated with MSA, 1 mg for the mice treated with 100 ng of sIL-4R, and 0.5 mg for mice treated with 1µg sIL-4R. No detectable difference in weight of lymph nodes was ascertainable for the mice treated with 2µg sIL-4R. Thus, IL-4R significantly ( $p < 0.5$  in all groups, using a two-tailed T test) suppressed the *in vivo* lymphoproliferative response in a dose dependent fashion relative to control mice.

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**Claims**

1. An isolated DNA sequence encoding a mammalian IL-4 receptor (IL-4R) or a fragment thereof.
- 30 2. An isolated DNA sequence encoding a polypeptide product of a procaryotic or eucaryotic host expression, the product having all or part of the primary structural conformation of a mammalian IL-4R and a biological activity of a mammalian IL-4R, or a fragment thereof.
3. A DNA sequence according to claim 1 or 2 which is any of:
  - (a) a cDNA clone having a nucleotide sequence derived from the coding region of a native  
 35 mammalian IL-4R gene or a fragment thereof;
  - (b) a DNA sequence capable of hybridization to the clones of (a) under moderately stringent conditions and which encode biologically active IL-4R or a fragment thereof; or
  - (c) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) or a fragment thereof and which encode biologically active IL-4R.
- 40 4. A DNA sequence according to any of claims 1 to 3, which encodes an amino acid sequence which is substantially similar to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.
5. A DNA sequence according to any of claims 1 to 4, which encodes an amino acid sequence which is substantially identical to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.
6. A recombinant expression vector comprising a DNA sequence according to any of claims 1 to 5.
- 45 7. A process for preparing a mammalian IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector according to claim 6 under conditions promoting expression.
8. A process for preparing a human IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector according to claim 6 under conditions promoting expression.
9. A population of eukaryotic cells which express more than  $10^4$  surface IL-4 receptors per cell.
- 50 10. A population of eukaryotic cells according to claim 9, which express more than  $10^5$  surface IL-4 receptors per cell.
11. A homogeneous biologically active mammalian IL-4 receptor composition.
12. A homogeneous biologically active mammalian IL-4 receptor composition according to claim 11, consisting essentially of murine IL-4 receptor, or human IL-4 receptor.
- 65 13. Mammalian IL-4 receptor or analog thereof having substantially similar biological activity.
14. A IL-4 receptor according to claim 13 which is in the form of a glycoprotein having a molecular weight of between about 110,000 and 150,000 M<sub>r</sub> by SDS-PAGE and a binding affinity ( $K_d$ ) for human IL-4 of from  $1-8 \times 10^9 \text{ M}^{-1}$ .

15. A IL-4 receptor according to claim 13 or 14 which has an N terminal amino acid sequence Met-Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.
16. A IL-4 receptor according to any of claims 13 to 15 wherein the transmembrane region and cytoplasmic domain of the native receptor have been deleted.
17. A IL-4 receptor according to any of claims 13 to 16 which is greater than about 80 percent similar to all or part of the sequence of amino acid residues 1-800 depicted in Figures 4A, 4B and 4C.
18. A IL-4 receptor according to any of claims 13 to 16 which is greater than about 80 percent similar to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.
19. A pharmaceutical composition for regulating immune response in a mammal, comprising an effective amount of a composition according to claim 11 or 12 and/or a receptor according to any of claims 13 to 18 and a suitable diluent or carrier.
20. A composition according to claim 19 having a specific binding activity of at least about 0.01 nanomole IL-4/nanomole IL-4 receptor.
21. A composition according to claim 19 or 20 consisting essentially of a substantially homogeneous protein composition comprising human IL-4 receptor in the form of a glycoprotein having a binding affinity ( $K_d$ ) for human IL-4 of about  $1-8 \times 10^9 M^{-1}$  and the N-terminal amino acid sequence Met-Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.
22. A composition according to claim 11 or 12 wherein the IL-4 receptor is capable of retaining IL-4 binding activity when bound to nitrocellulose.
23. An assay method for detection of IL-4 or IL-4 receptor molecules or the interaction thereof, comprising using a composition according to any of claims 11, 12 or 19 to 21 or a receptor as claimed in any of claims 13 to 18.
24. A mammalian IL-4 receptor for use in human or veterinary medicine.
25. The use of IL-4 receptor in the preparation of medicament for regulating immune responses in a mammal.
26. The use of claim 25, wherein the IL-4 receptor and the mammal to be treated is a human.
27. Antibodies immunoreactive with mammalian IL-4 receptors.
28. A host transfected with a DNA sequence as claimed in any of claims 1 to 5 and/or a vector as claimed in claim 6.
29. RNA substantially complementary to the DNA as claimed in any of claims 1 to 5.
30. A fusion protein comprising a polypeptide sequence coupled to a mammalian IL-4 receptor as claimed in any of claims 13 to 18.

#### Claims for the following Contracting State: GR

1. An isolated DNA sequence encoding a mammalian IL-4 receptor (IL-4R) or a fragment thereof.
2. An isolated DNA sequence encoding a polypeptide product of a procaryotic or eucaryotic host expression, the product having all or part of the primary structural conformation of a mammalian IL-4R and a biological activity of a mammalian IL-4R, or a fragment thereof.
3. A DNA sequence according to claim 1 or 2 which is any of:
- a cDNA clone having a nucleotide sequence derived from the coding region of a native mammalian IL-4R gene or a fragment thereof;
  - a DNA sequence capable of hybridization to the clones of (a) under moderately stringent conditions and which encode biologically active IL-4R or a fragment thereof; or
  - a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) or a fragment thereof and which encode biologically active IL-4R.
4. A DNA sequence according to any of claims 1 to 3, which encodes an amino acid sequence which is substantially similar to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.
5. A DNA sequence according to any of claims 1 to 4, which encodes an amino acid sequence which is substantially identical to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.
6. A recombinant expression vector comprising a DNA sequence according to any of claims 1 to 5.
7. A process for preparing a mammalian IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector according to claim 6 under conditions promoting expression.
8. A process for preparing a human IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector according to claim 6 under conditions promoting expression.
9. A population of eukaryotic cells which express more than  $10^4$  surface IL-4 receptors per cell.
10. A population of eukaryotic cells according to claim 9, which express more than  $10^5$  surface IL-4 receptors per cell.

11. A process for the preparation of a homogeneous biologically active mammalian IL-4 receptor composition, the process comprising adding a substance to the IL-4 receptor.

12. A process according to claim 11 wherein the receptor is essentially murine IL-4 receptor, or human IL-4 receptor.

13. A process for the preparation of mammalian IL-4 receptor or analog thereof having substantially similar biological activity, the process comprising coupling successive nucleotides together.

14. A process according to claim 13 wherein the receptor is in the form of a glycoprotein having a molecular weight of between about 110,000 and 150,000 M, by SDS-PAGE and a binding affinity ( $K_d$ ) for human IL-4 of from  $1-8 \times 10^9 M^{-1}$ .

15. A process according to claim 13 or 14 wherein the receptor has an N terminal amino acid sequence Met-Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.

16. A process according to any of claims 13 to 15 wherein the transmembrane region and cytoplasmic domain of the native receptor have been deleted.

17. A process according to any of claims 13 to 16 wherein the receptor is greater than about 80 percent similar to all or part of the sequence of amino acid residues 1-800 depicted in Figures 4A, 4B and 4C.

18. A process according to any of claims 13 to 16 wherein the receptor is greater than about 80 percent similar to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.

19. A process for the preparation of a pharmaceutical composition for regulating immune response in a mammal, the process comprising admixing an effective amount of a composition prepared in claim 11 or 12 and/or a receptor prepared in any of claims 13 to 18 and a suitable diluent or carrier.

20. A process according to claim 19 wherein the receptor has a specific binding activity of at least about 0.01 nanomole IL-4/nanomole IL-4 receptor.

21. A process according to claim 19 or 20 wherein the composition consists essentially of a substantially homogeneous protein composition comprising human IL-4 receptor in the form of a glycoprotein having a binding affinity ( $K_d$ ) for human IL-4 of about  $1-8 \times 10^9 M^{-1}$ , and the N-terminal amino acid sequence Met-Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.

22. A process according to claim 11 or 12 wherein the IL-4 receptor is capable of retaining IL-4 binding activity when bound to nitrocellulose.

23. An assay method for detection of IL-4 or IL-4 receptor molecules or the interaction thereof, comprising using a composition prepared in any of claims 11, 12 or 19 to 21 or a receptor as claimed in any of claims 13 to 18.

24. A process for the preparation of antibodies immunoreactive with IL-4 receptor, the process comprising either (a) culturing a hybridoma cell expressing the antibodies and harvesting the antibodies, or (b) harvesting antibodies immunoreactive with IL-4 receptor from an appropriately immunised animal.

25. The use of IL-4 receptor in the preparation of medicament for regulating immune responses in a mammal.

26. The use of claim 25, wherein the IL-4 receptor and the mammal to be treated is a human.

27. Antibodies immunoreactive with mammalian IL-4 receptors.

28. A host transfected with a DNA sequence as prepared in any of claims 1 to 5 and/or a vector as claimed in claim 6.

29. RNA substantially complementary to the DNA as claimed in any of claims 1 to 5.

30. A process for the preparation of a fusion protein comprising a polypeptide sequence coupled to a mammalian IL-4 receptor as claimed in any of claims 13 to 18, the process comprising coupling successive nucleotides together.

Claims for the following Contracting State: ES

1. A process for the preparation of an isolated DNA sequence encoding a mammalian IL-4 receptor (IL-4R) or a fragment thereof, comprising coupling successive nucleotides together or ligating oligonucleotides.

2. A process of the preparation of an isolated DNA sequence encoding a polypeptide product of a procaryotic or eucaryotic host expression, the product having all or part of the primary structural conformation of a mammalian IL-4R and a biological activity of a mammalian IL-4R, or a fragment thereof, comprising coupling successive nucleotides together or ligating oligonucleotides.

3. A process according to claim 1 or 2 which is any of:

(a) a cDNA clone having a nucleotide sequence derived from the coding region of a native mammalian IL-4R gene or a fragment thereof;

(b) a DNA sequence capable of hybridization to the clones of (a) under moderately stringent conditions and which encode biologically active IL-4R or a fragment thereof; or

(c) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) or a fragment thereof and which encode biologically active IL-4R.

4. A process according to any of claims 1 to 3, which encodes an amino acid sequence which is substantially similar to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.

5 5. A process according to any of claims 1 to 4, which encodes an amino acid sequence which is substantially identical to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.

6. A process for the preparation of a recombinant expression vector comprising ligating a DNA sequence according to any of claims 1 to 5 with an expression vector DNA.

7. A process for preparing a mammalian IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector prepared according to claim 6 under conditions promoting expression.

8. A process for preparing a human IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector prepared according to claim 6 under conditions promoting expression.

9. A process for the population of eukaryotic cells which express more than  $10^4$  surface IL-4 receptors per cell, comprising culturing the cells.

10 10. A process according to claim 9, which express more than  $10^5$  surface IL-4 receptors per cell.

11. A process for the preparation of a homogeneous biologically active mammalian IL-4 receptor composition, the process comprising adding a substance to the IL-4 receptor.

12. A process according to claim 11 wherein the receptor is essentially murine IL-4 receptor, or human IL-4 receptor.

13. A process for the preparation of mammalian IL-4 receptor or analog thereof having substantially similar biological activity, the process comprising coupling successive amino acid residues together.

14. A process according to claim 13 which is in the form of a glycoprotein having a molecular weight of between about 110,000 and 150,000 M, by SDS-PAGE and a binding affinity ( $K_D$ ) for human IL-4 of from  $1-8 \times 10^9 M^{-1}$ .

15 15. A process according to claim 13 or 14 which has an N terminal amino acid sequence Met-Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.

16. A process according to any of claims 13 to 15 wherein the transmembrane region and cytoplasmic domain of the native receptor have been deleted.

17. A process according to any of claims 13 to 16 which is greater than about 80 percent similar to all or part of the sequence of amino acid residues 1-800 depicted in Figures 4A, 4B and 4C.

18. A process according to any of claims 13 to 16 which is substantially similar or substantially identical to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.

19. A process for the preparation of a pharmaceutical composition for regulating immune response in a mammal, the process comprising admixing an effective amount of a composition prepared in claim 11 or 12 and/or a receptor prepared in any of claims 13 to 18 with a suitable diluent or carrier.

20. A process according to claim 19 wherein the receptor has a specific binding activity of at least about 0.01 nanomole IL-4/nanomole IL-4 receptor.

21. A process according to claim 19 or 20 wherein the composition consists essentially of a substantially homogeneous protein composition comprising human IL-4 receptor in the form of a glycoprotein having a binding affinity ( $K_D$ ) for human IL-4 of about  $1-8 \times 10^9 M^{-1}$ , and the N-terminal amino acid sequence Met-Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.

22. A process according to claim 11 or 12 wherein the IL-4 receptor is capable of retaining IL-4 binding activity when bound to nitrocellulose.

23. An assay for the detection of IL-4 or IL-4 receptor molecules or the interaction thereof, comprising using a soluble mammalian IL-4 receptor, IL-4 receptor subunit, or substantially similar or identical IL-4 receptor analog produced by recombinant cell culture and having a specific binding activity of at least 0.01 nmole IL-4/nmole IL-4 receptor.

24. The use of IL-4 receptor in the preparation of medicament for regulating immune responses in a mammal.

25 25. The use of claim 24, wherein the IL-4 receptor and the mammal to be treated is a human.

27. A process for the preparation of antibodies immunoreactive with IL-4 receptor, the process comprising either (a) culturing a hybridoma cell expressing the antibodies and harvesting the antibodies, or (b) harvesting antibodies immunoreactive with IL-4 receptor from an appropriately immunised animal.

28. A host transfected with a DNA sequence as prepared in any of claims 1 to 5 and/or a vector as claimed in claim 6.

29. RNA substantially complementary to the DNA as claimed in any of claims 1 to 5.

30. A process for the preparation of a fusion protein comprising a polypeptide sequence coupled to a mammalian IL-4 receptor as claimed in any of claims 13 to 18, the process comprising coupling successive

amino acids together.

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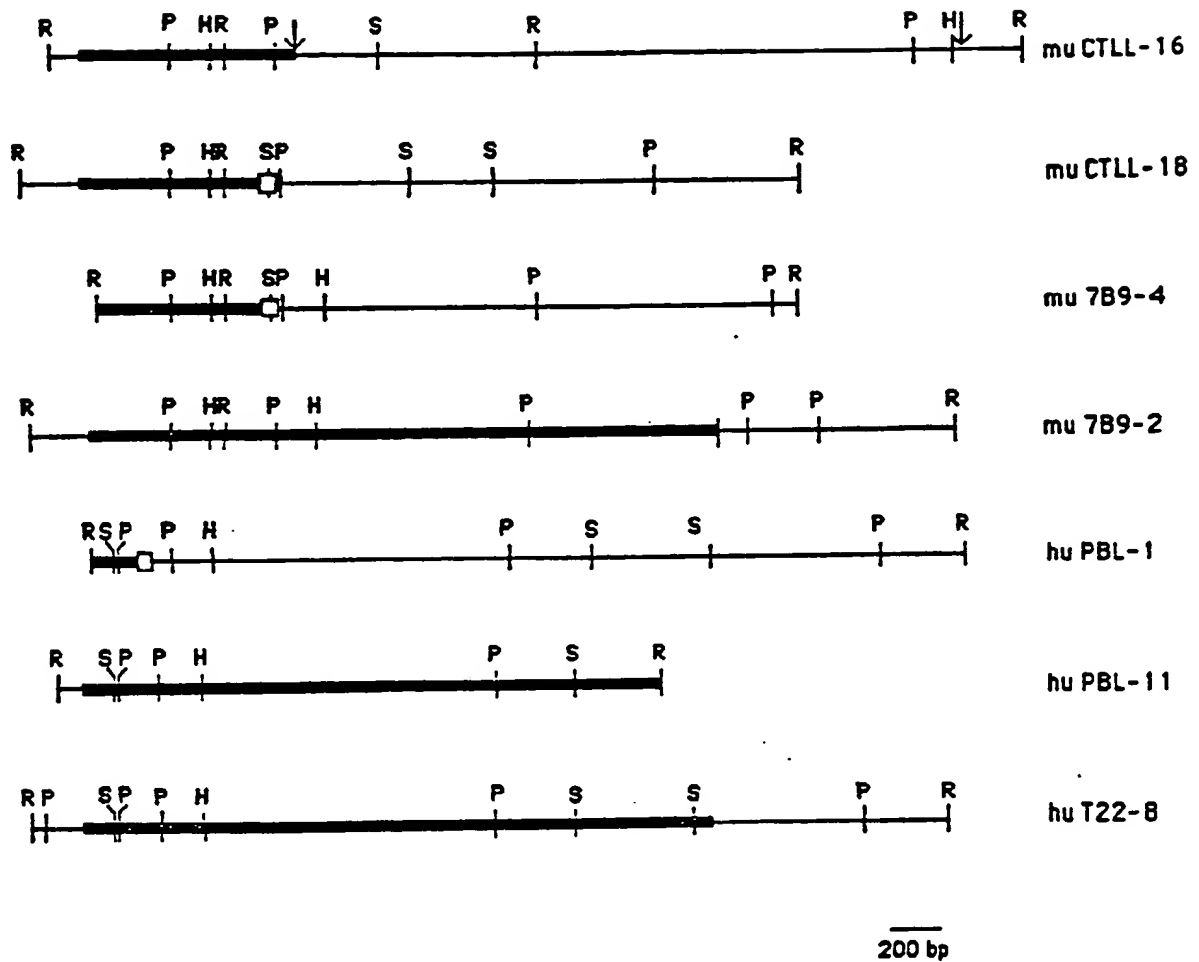
**FIGURE 1**

FIGURE 2A

ATG	GGG	CGG	CTT	TGC	ACC	AAG	TTC	CTG	ACC	TCT	GTG	GGC	TGT	CTG	-31
Met	Gly	Arg	Leu	Cys	Thr	Lys	Phe	Leu	Thr	Ser	Val	Gly	Cys	Leu	-11
ATT	TTG	CTG	TTG	GTG	ACT	GGA	TCT	GGG	AGC	ATC	AAG	GTC	CTG	GGT	15
Ile	Leu	Leu	Leu	Val	Thr	Gly	Ser	Gly	Ser	Ile	Lys	Val	Leu	Gly	5
GAG	CCC	ACC	TGC	TTC	TCT	GAC	TAC	ATC	CGC	ACT	TCC	ACG	TGT	GAG	60
Glu	Pro	Thr	Cys	Phe	Ser	Asp	Tyr	Ile	Arg	Thr	Ser	Thr	Cys	Glu	20
TGG	TTC	CTG	GAT	AGC	GCT	GTG	GAC	TGC	AGT	TCT	CAG	CTC	TGC	CTA	105
Trp	Phe	Leu	Asp	Ser	Ala	Val	Asp	Cys	Ser	Ser	Gln	Leu	Cys	Leu	35
CAC	TAC	AGG	CTG	ATG	TTC	TTC	GAG	TTC	TCT	GAA	AAC	CTC	ACA	TGC	150
His	Tyr	Arg	Leu	Met	Phe	Phe	Glu	Phe	Ser	Glu	Asn	Leu	Thr	Cys	50
ATC	CCG	AGG	AAC	AGT	GCC	AGC	ACT	GTG	TGT	GTG	TGC	CAC	ATG	GAA	195
Ile	Pro	Arg	Asn	Ser	Ala	Ser	Thr	Val	Cys	Val	Cys	His	Met	Glu	65
ATG	AAT	AGG	CCG	GTC	CAA	TCA	GAC	AGA	TAC	CAG	ATG	GAA	CTG	TGG	240
Met	Asn	Arg	Pro	Val	Gln	Ser	Asp	Arg	Tyr	Gln	Met	Glu	Leu	Trp	80
GCT	GAG	CAC	AGA	CAG	CTG	TGG	CAG	GGC	TCC	TTC	AGC	CCC	AGT	GGT	285
Ala	Glu	His	Arg	Gln	Leu	Trp	Gln	Gly	Ser	Phe	Ser	Pro	Ser	Gly	95
AAT	GTG	AAG	CCC	CTA	GCT	CCA	GAC	AAC	CTC	ACA	CTC	CAC	ACC	AAT	330
Asn	Val	Lys	Pro	Leu	Ala	Pro	Asp	Asn	Leu	Thr	Leu	His	Thr	Asn	110
GTG	TCC	GAC	GAA	TGG	CTG	CTG	ACC	TGG	AAT	AAC	CTG	TAC	CCA	TCG	375
Val	Ser	Asp	Glu	Trp	Leu	Leu	Thr	Trp	Asn	Asn	Leu	Tyr	Pro	Ser	125
AAC	AAC	TTA	CTG	TAC	AAA	GAC	CTC	ATC	TCC	ATG	GTC	AAC	ATC	TCC	420
Asn	Asn	Leu	Leu	Tyr	Lys	Asp	Leu	Ile	Ser	Met	Val	Asn	Ile	Ser	140
AGA	GAG	GAC	AAC	CCT	GCA	GAA	TTC	ATA	GTC	TAT	AAT	GTG	ACC	TAC	465
Arg	Glu	Asp	Asn	Pro	Ala	Glu	Phe	Ile	Val	Tyr	Asn	Val	Thr	Tyr	155
AAG	GAA	CCC	AGG	CTG	AGC	TTC	CCG	ATC	AAC	ATC	CTG	ATG	TCA	GGG	510
Lys	Glu	Pro	Arg	Leu	Ser	Phe	Pro	Ile	Asn	Ile	Leu	Met	Ser	Gly	170
GTC	TAC	TAT	ACG	GCG	CGT	GTG	AGG	GTC	AGA	TCC	CAG	ATA	CTC	ACT	555
Val	Tyr	Tyr	Thr	Ala	Arg	Val	Arg	Val	Arg	Ser	Gln	Ile	Leu	Thr	185
GGC	ACC	TGG	AGT	GAG	TGG	AGT	CCT	AGC	ATC	ACG	TGG	TAC	AAC	CAC	600
Gly	Thr	Trp	Ser	Glu	Trp	Ser	Pro	Ser	Ile	Thr	Trp	Tyr	Asn	His	200
TTC	CAG	CTG	CCC	CTG	ATA	CAG	CGC	CTT	CCA	CTG	GGG	GTC	ACC	ATC	645
Phe	Gln	Leu	Pro	Leu	Ile	Gln	Arg	Leu	Pro	Leu	Gly	Val	Thr	Ile	215
TCC	TGC	CTC	TGC	ATC	CCG	TTG	TTT	TGC	CTG	TTC	TGT	TAC	TTC	AGC	690
Ser	Cys	Leu	Cys	Ile	Pro	Leu	Phe	Cys	Leu	Phe	Cys	Tyr	Phe	Ser	230
ATT	ACC	AAG	ATT	AAG	AAG	ATA	TGG	TGG	GAC	CAG	ATT	CCC	ACC	CCA	735
Ile	Thr	Lys	Ile	Lys	Lys	Ile	Trp	Trp	Asp	Gln	Ile	Pro	Thr	Pro	245

FIGURE 2B

GCA	CGC	AGT	CCC	TTG	GTG	GCC	ATC	ATC	ATT	CAG	GAT	GCA	CAG	GTG	780
Ala	Arg	Ser	Pro	Leu	Val	Ala	Ile	Ile	Ile	Gln	Asp	Ala	Gln	Val	260
CCC	CTC	TGG	GAT	AAG	CAG	ACC	CGA	AGC	CAG	GAG	TCA	ACC	AAG	TAC	825
Pro	Leu	Trp	Asp	Lys	Gln	Thr	Arg	Ser	Gln	Glu	Ser	Thr	Lys	Tyr	275
CCG	CAC	TGG	AAA	ACT	TGT	CTA	GAC	AAG	CTG	CTG	CCT	TGC	TTG	CTG	870
Pro	His	Trp	Lys	Thr	Cys	Leu	Asp	Lys	Leu	Leu	Pro	Cys	Leu	Leu	290
AAG	CAC	AGA	GTA	AAG	AAG	AAG	ACA	GAC	TTC	CCG	AAG	GCT	GCC	CCA	915
Lys	His	Arg	Val	Lys	Lys	Lys	Thr	Asp	Phe	Pro	Lys	Ala	Ala	Pro	305
ACC	AAG	TCT	CTC	CAG	AGT	CCT	GGA	AAG	GCA	GGC	TGG	TGT	CCC	ATG	960
Thr	Lys	Ser	Leu	Gln	Ser	Pro	Gly	Lys	Ala	Gly	Trp	Cys	Pro	Met	320
GAG	GTC	AGC	AGG	ACC	GTC	CTC	TGG	CCA	GAG	AAT	GTT	AGT	GTC	AGT	1005
Glu	Val	Ser	Arg	Thr	Val	Leu	Trp	Pro	Glu	Asn	Val	Ser	Val	Ser	335
GTG	GTG	CGC	TGT	ATG	GAG	CTG	TTT	GAG	GCC	CCA	GTA	CAG	AAT	GTG	1050
Val	Val	Arg	Cys	Met	Glu	Leu	Phe	Glu	Ala	Pro	Val	Gln	Asn	Val	350
GAG	GAG	GAA	GAA	GAT	GAG	ATA	GTC	AAA	GAG	GAC	CTG	AGC	ATG	TCA	1095
Glu	Glu	Glu	Glu	Asp	Glu	Ile	Val	Lys	Glu	Asp	Leu	Ser	Met	Ser	365
CCT	GAG	AAC	AGC	GGA	GGC	TGC	GGC	TTC	CAG	GAG	AGC	CAG	GCA	GAC	1140
Pro	Glu	Asn	Ser	Gly	Gly	Cys	Gly	Phe	Gln	Glu	Ser	Gln	Ala	Asp	380
ATC	ATG	GCT	CGG	CTC	ACT	GAG	AAC	CTG	TTT	TCC	GAC	TTG	TTG	GAG	1185
Ile	Met	Ala	Arg	Leu	Thr	Glu	Asn	Leu	Phe	Ser	Asp	Leu	Leu	Glu	395
GCT	GAG	AAT	GGG	GGC	CTT	GGC	CAG	TCA	GCC	TTG	GCA	GAG	TCA	TGC	1230
Ala	Glu	Asn	Gly	Gly	Leu	Gly	Gln	Ser	Ala	Leu	Ala	Glu	Ser	Cys	410
TCC	CCT	CTG	CCT	TCA	GGA	AGT	GGG	CAG	GCT	TCT	GTA	TCC	TGG	GCC	1275
Ser	Pro	Leu	Pro	Ser	Gly	Ser	Gly	Gln	Ala	Ser	Val	Ser	Trp	Ala	425
TGC	CTC	CCC	ATG	GGG	CCC	AGT	GAG	GAG	GCC	ACA	TGC	CAG	GTC	ACA	1320
Cys	Leu	Pro	Met	Gly	Pro	Ser	Glu	Glu	Ala	Thr	Cys	Gln	Val	Thr	440
GAG	CAG	CCT	TCA	CAC	CCA	GGC	CCT	CTT	TCA	GGC	AGC	CCA	GCC	CAG	1365
Glu	Gln	Pro	Ser	His	Pro	Gly	Pro	Leu	Ser	Gly	Ser	Pro	Ala	Gln	455
AGT	GCA	CCT	ACT	CTG	GCT	TGC	ACG	CAG	GTC	CCA	CTT	GTC	CTT	GCA	1410
Ser	Ala	Pro	Thr	Leu	Ala	Cys	Thr	Gln	Val	Pro	Leu	Val	Leu	Ala	470
GAC	AAT	CCT	GCC	TAC	CGG	AGT	TTT	AGT	GAC	TGC	TGT	AGC	CCG	GCC	1455
Asp	Asn	Pro	Ala	Tyr	Arg	Ser	Phe	Ser	Asp	Cys	Cys	Ser	Pro	Ala	485
CCA	AAT	CCT	GGA	GAG	CTG	GCT	CCA	GAG	CAG	CAG	CAG	GCT	GAT	CAT	1500
Pro	Asn	Pro	Gly	Glu	Leu	Ala	Pro	Glu	Gln	Gln	Gln	Ala	Asp	His	500
CTG	GAA	GAA	GAG	GAG	CCT	CCA	AGC	CCG	GCT	GAC	CCC	CAT	TCT	TCA	1545
Leu	Glu	Glu	Glu	Glu	Pro	Pro	Ser	Pro	Ala	Asp	Pro	His	Ser	Ser	515

FIGURE 2C

GGG	CCA	CCA	ATG	CAG	CCA	GTG	GAG	AGC	TGG	GAG	CAG	ATC	CTT	CAC	1590
Gly	Pro	Pro	Met	Gln	Pro	Val	Glu	Ser	Trp	Glu	Gln	Ile	Leu	His	530
ATG	AGT	GTC	CTG	CAG	CAT	GGG	GCA	GCT	GCT	GGC	TCC	ACC	CCA	GCC	1635
Met	Ser	Val	Leu	Gln	His	Gly	Ala	Ala	Ala	Gly	Ser	Thr	Pro	Ala	545
CCT	GCC	GGT	GGC	TAC	CAG	GAG	TTT	GTG	CAG	GCA	GTG	AAG	CAG	GGT	1680
Pro	Ala	Gly	Gly	Tyr	Gln	Glu	Phe	Val	Gln	Ala	Val	Lys	Gln	Gly	560
GCC	GCC	CAG	GAT	CCT	GGG	GTG	CCT	GGT	GTC	AGG	CCT	TCT	GGA	GAC	1725
Ala	Ala	Gln	Asp	Pro	Gly	Val	Pro	Gly	Val	Arg	Pro	Ser	Gly	Asp	575
CCC	GGT	TAC	AAG	GCC	TTC	TCG	AGC	CTG	CTC	AGC	AGC	AAT	GGC	ATC	1770
Pro	Gly	Tyr	Lys	Ala	Phe	Ser	Ser	Leu	Leu	Ser	Ser	Asn	Gly	Ile	590
CGC	GGG	GAC	ACA	GCA	GCA	GCG	GGG	ACT	GAC	GAT	GGG	CAT	GGA	GGC	1815
Arg	Gly	Asp	Thr	Ala	Ala	Ala	Gly	Thr	Asp	Asp	Gly	His	Gly	Gly	605
TAC	AAG	CCC	TTC	CAG	AAT	CCT	GTT	CCT	AAC	CAG	TCC	CCT	AGC	TCC	1860
Tyr	Lys	Pro	Phe	Gln	Asn	Pro	Val	Pro	Asn	Gln	Ser	Pro	Ser	Ser	620
GTG	CCC	TTA	TTT	ACT	TTC	GGA	CTA	GAC	ACG	GAG	CTG	TCA	CCC	AGT	1905
Val	Pro	Leu	Phe	Thr	Phe	Gly	Leu	Asp	Thr	Glu	Leu	Ser	Pro	Ser	635
CCT	CTG	AAC	TCA	GAC	CCA	CCC	AAA	AGC	CCC	CCA	GAA	TGC	CTT	GGT	1950
Pro	Leu	Asn	Ser	Asp	Pro	Pro	Lys	Ser	Pro	Pro	Glu	Cys	Leu	Gly	650
CTG	GAG	CTG	GGG	CTC	AAA	GGA	GGT	GAC	TGG	GTG	AAG	GCC	CCT	CCT	1995
Leu	Glu	Leu	Gly	Leu	Lys	Gly	Gly	Asp	Trp	Val	Lys	Ala	Pro	Pro	665
CCT	GCA	GAT	GAG	GTG	CCC	AAG	CCC	TTT	GGG	GAT	GAC	CTG	GGC	TTT	2040
Pro	Ala	Asp	Glu	Val	Pro	Lys	Pro	Phe	Gly	Asp	Asp	Leu	Gly	Phe	680
GGT	ATT	GTG	TAC	TCG	TCC	CTC	ACT	TGC	CAC	TTG	TGT	GGC	CAC	CTG	2085
Gly	Ile	Val	Tyr	Ser	Ser	Leu	Thr	Cys	His	Leu	Cys	Gly	His	Leu	695
AAG	CAA	CAC	CAC	AGC	CAG	GAG	GAA	GGT	GGC	CAG	AGC	CCC	ATC	GTT	2130
Lys	Gln	His	His	Ser	Gln	Glu	Glu	Gly	Gly	Gln	Ser	Pro	Ile	Val	710
GCT	AGC	CCT	GGC	TGT	GGC	TGC	TGC	TAC	GAT	GAC	AGA	TCA	CCA	TCC	2175
Ala	Ser	Pro	Gly	Cys	Gly	Cys	Cys	Tyr	Asp	Asp	Arg	Ser	Pro	Ser	725
CTG	GGG	AGC	CTC	TCG	GGG	GCC	TTG	GAA	AGC	TGT	CCT	GAG	GGA	ATA	2220
Leu	Gly	Ser	Leu	Ser	Gly	Ala	Leu	Glu	Ser	Cys	Pro	Glu	Gly	Ile	740
CCA	CCA	GAA	GCC	AAC	CTC	ATG	TCA	GCA	CCC	AAG	ACA	CCC	TCA	AAC	2265
Pro	Pro	Glu	Ala	Asn	Leu	Met	Ser	Ala	Pro	Lys	Thr	Pro	Ser	Asn	755
TTG	TCA	GGG	GAG	GGC	AAG	GGC	CCT	GGT	CAC	TCT	CCT	GTT	CCC	AGC	2310
Leu	Ser	Gly	Glu	Gly	Lys	Gly	Pro	Gly	His	Ser	Pro	Val	Pro	Ser	770
CAG	ACG	ACC	GAG	GTG	CCT	GTG	GGC	GCC	CTG	GGC	ATT	GCT	GTT	TCT	2355
Gln	Thr	Thr	Glu	Val	Pro	Val	Gly	Ala	Leu	Gly	Ile	Ala	Val	Ser	785

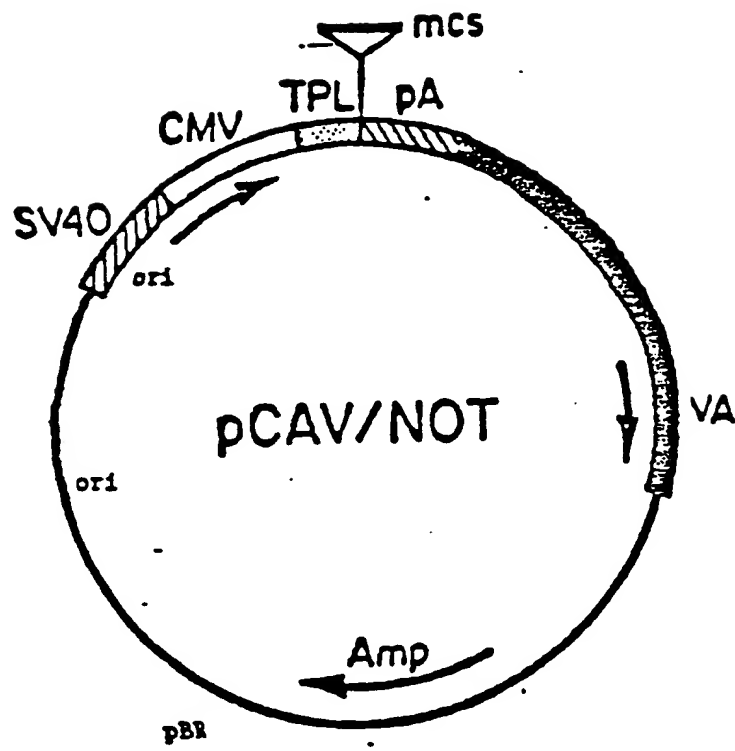


Fig. 3

**FIGURE 4A**

ATG	GGG	TGG	CTT	TGC	TCT	GGG	CTC	CTG	TTC	CCT	GTG	AGC	TGC	CTG	-31
Met	Gly	Trp	Leu	Cys	Ser	Gly	Leu	Leu	Phe	Pro	Val	Ser	Cys	Leu	-11
GTC	CTG	CTG	CAG	GTG	GCA	AGC	TCT	GGG	AAC	ATG	AAG	GTC	TTG	CAG	15
Val	Leu	Leu	Gln	Val	Ala	Ser	Ser	Gly	Asn	<u>Met</u>	Lys	Val	Leu	Gln	5
GAG	CCC	ACC	TGC	GTC	TCC	GAC	TAC	ATG	AGC	ATC	TCT	ACT	TGC	GAG	60
Glu	Pro	Thr	Cys	Val	Ser	Asp	Tyr	Met	Ser	Ile	Ser	Thr	Cys	Glu	20
TGG	AAG	ATG	AAT	GGT	CCC	ACC	AAT	TGC	AGC	ACC	GAG	CTC	CGC	CTG	105
Trp	Lys	Met	Asn	Gly	Pro	Thr	Asn	Cys	Ser	Thr	Glu	Leu	Arg	Leu	35
TTG	TAC	CAG	CTG	GTT	TTT	CTG	CTC	TCC	GAA	GCC	CAC	ACG	TGT	ATC	150
Leu	Tyr	Gln	Leu	Val	Phe	Leu	Leu	Ser	Glu	Ala	His	Thr	Cys	Ile	50
CCT	GAG	AAC	AAC	GGA	GGC	GCG	GGG	TGC	GTG	TGC	CAC	CTG	CTC	ATG	195
Pro	Glu	Asn	Asn	Gly	Gly	Ala	Gly	Cys	Val	Cys	His	Leu	Leu	Met	65
GAT	GAC	GTG	GTC	AGT	GCG	GAT	AAC	TAT	ACA	CTG	GAC	CTG	TGG	GCT	240
Asp	Asp	Val	Val	Ser	Ala	Asp	Asn	Tyr	Thr	Leu	Asp	Leu	Trp	Ala	80
GGG	CAG	CAG	CTG	CTG	TGG	AAG	GGC	TCC	TTC	AAG	CCC	AGC	GAG	CAT	285
Gly	Gln	Gln	Leu	Leu	Trp	Lys	Gly	Ser	Phe	Lys	Pro	Ser	Glu	His	95
GTG	AAA	CCC	AGG	GCC	CCA	GGA	AAC	CTG	ACA	GTT	CAC	ACC	AAT	GTC	330
Val	Lys	Pro	Arg	Ala	Pro	Gly	Asn	Leu	Thr	Val	His	Thr	Asn	Val	110
TCC	GAC	ACT	CTG	CTG	CTG	ACC	TGG	AGC	AAC	CCG	TAT	CCC	CCT	GAC	375
Ser	Asp	Thr	Leu	Leu	Leu	Thr	Trp	Ser	Asn	Pro	Tyr	Pro	Pro	Asp	125
AAT	TAC	CTG	TAT	AAT	CAT	CTC	ACC	TAT	GCA	GTC	AAC	ATT	TGG	AGT	420
Asn	Tyr	Leu	Tyr	Asn	His	Leu	Thr	Tyr	Ala	Val	Asn	Ile	Trp	Ser	140
GAA	AAC	GAC	CCG	GCA	GAT	TTC	AGA	ATC	TAT	AAC	GTG	ACC	TAC	CTA	465
Glu	Asn	Asp	Pro	Ala	Asp	Phe	Arg	Ile	Tyr	Asn	Val	Thr	Tyr	Leu	155
GAA	CCC	TCC	CTC	CGC	ATC	GCA	GCC	AGC	ACC	CTG	AAG	TCT	GGG	ATT	510
Glu	Pro	Ser	Leu	Arg	Ile	Ala	Ala	Ser	Thr	Leu	Lys	Ser	Gly	Ile	170
TCC	TAC	AGG	GCA	CGG	GTG	AGG	GCC	TGG	GCT	CAG	TGC	TAT	AAC	ACC	555
Ser	Tyr	Arg	Ala	Arg	Val	Arg	Ala	Trp	Ala	Gln	Cys	Tyr	Asn	Thr	185
ACC	TGG	AGT	GAG	TGG	AGC	CCC	AGC	ACC	AAG	TGG	CAC	AAC	TCC	TAC	600
Thr	Trp	Ser	Glu	Trp	Ser	Pro	Ser	Thr	Lys	Trp	His	Asn	Ser	Tyr	200
AGG	GAG	CCC	TTC	GAG	CAG	CAC	CTC	CTG	CTG	GGC	GTC	AGC	GTT	TCC	645
Arg	Glu	Pro	Phe	Glu	Gln	His	<u>Leu</u>	<u>Leu</u>	<u>Leu</u>	<u>Gly</u>	<u>Val</u>	<u>Ser</u>	<u>Val</u>	<u>Ser</u>	215
TGC	ATT	GTC	ATC	CTG	GCC	GTC	TGC	CTG	TTG	TGC	TAT	GTC	AGC	ATC	690
<u>Cys</u>	<u>Ile</u>	<u>Val</u>	<u>Ile</u>	<u>Leu</u>	<u>Ala</u>	<u>Val</u>	<u>Cys</u>	<u>Leu</u>	<u>Leu</u>	<u>Cys</u>	<u>Tyr</u>	<u>Val</u>	<u>Ser</u>	<u>Ile</u>	230
ACC	AAG	ATT	AAG	AAA	GAA	TGG	TGG	GAT	CAG	ATT	CCC	AAC	CCA	GCC	735
Thr	Lys	Ile	Lys	Lys	Glu	Trp	Trp	Asp	Gln	Ile	Pro	Asn	Pro	Ala	245

FIGURE 4B

CGC	AGC	CGC	CTC	GTG	GCT	ATA	ATA	ATC	CAG	GAT	GCT	CAG	GGG	TCA	780
Arg	Ser	Arg	Leu	Val	Ala	Ile	Ile	Ile	Gln	Asp	Ala	Gln	Gly	Ser	260
CAG	TGG	GAG	AAG	CGG	TCC	CGA	GGC	CAG	GAA	CCA	GCC	AAG	TGC	CCA	825
Gln	Trp	Glu	Lys	Arg	Ser	Arg	Gly	Gln	Glu	Pro	Ala	Lys	Cys	Pro	275
CAC	TGG	AAG	AAT	TGT	CTT	ACC	AAG	CTC	TTG	CCC	TGT	TTT	CTG	GAG	870
His	Trp	Lys	Asn	Cys	Leu	Thr	Lys	Leu	Leu	Pro	Cys	Phe	Leu	Glu	290
CAC	AAC	ATG	AAA	AGG	GAT	GAA	GAT	CCT	CAC	AAG	GCT	GCC	AAA	GAG	915
His	Asn	Met	Lys	Arg	Asp	Glu	Asp	Pro	His	Lys	Ala	Ala	Lys	Glu	305
ATG	CCT	TTC	CAG	GGC	TCT	GGA	AAA	TCA	GCA	TGG	TGC	CCA	GTG	GAG	960
Met	Pro	Phe	Gln	Gly	Ser	Gly	Lys	Ser	Ala	Trp	Cys	Pro	Val	Glu	320
ATC	AGC	AAG	ACA	GTC	CTC	TGG	CCA	GAG	AGC	ATC	AGC	GTG	GTG	CGA	1005
Ile	Ser	Lys	Thr	Val	Leu	Trp	Pro	Glu	Ser	Ile	Ser	Val	Val	Arg	335
TGT	GTG	GAG	TTG	TTT	GAG	GCC	CCG	GTG	GAG	TGT	GAG	GAG	GAG	GAG	1050
Cys	Val	Glu	Leu	Phe	Glu	Ala	Pro	Val	Glu	Cys	Glu	Glu	Glu	Glu	350
GAG	GTA	GAG	GAA	GAA	AAA	GGG	AGC	TTC	TGT	GCA	TCG	CCT	GAG	AGC	1095
Glu	Val	Glu	Glu	Glu	Lys	Gly	Ser	Phe	Cys	Ala	Ser	Pro	Glu	Ser	365
AGC	AGG	GAT	GAC	TTC	CAG	GAG	GGA	AGG	GAG	GGC	ATT	GTG	GCC	CGG	1140
Ser	Arg	Asp	Asp	Phe	Gln	Glu	Gly	Arg	Glu	Gly	Ile	Val	Ala	Arg	380
CTA	ACA	GAG	AGC	CTG	TTC	CTG	GAC	CTG	CTC	GGA	GAG	GAG	AAT	GGG	1185
Leu	Thr	Glu	Ser	Leu	Phe	Leu	Asp	Leu	Leu	Gly	Glu	Glu	Asn	Gly	395
GGC	TTT	TGC	CAG	CAG	GAC	ATG	GGG	GAG	TCA	TGC	CTT	CTT	CCA	CCT	1230
Gly	Phe	Cys	Gln	Gln	Asp	Met	Gly	Glu	Ser	Cys	Leu	Leu	Pro	Pro	410
TCG	GGA	AGT	ACG	AGT	GCT	CAC	ATG	CCC	TGG	GAT	GAG	TTC	CCA	AGT	1275
Ser	Gly	Ser	Thr	Ser	Ala	His	Met	Pro	Trp	Asp	Glu	Phe	Pro	Ser	425
GCA	GGG	CCC	AAG	GAG	GCA	CCT	CCC	TGG	GGC	AAG	GAG	CAG	CCT	CTC	1320
Ala	Gly	Pro	Lys	Glu	Ala	Pro	Pro	Trp	Gly	Lys	Glu	Gln	Pro	Leu	440
CAC	CTG	GAG	CCA	AGT	CCT	CCT	GCC	AGC	CCG	ACC	CAG	AGT	CCA	GAC	1365
His	Leu	Glu	Pro	Ser	Pro	Pro	Ala	Ser	Pro	Thr	Gln	Ser	Pro	Asp	455
AAC	CTG	ACT	TGC	ACA	GAG	ACG	CCC	CTC	GTC	ATC	GCA	GGC	AAC	CCT	1410
Asn	Leu	Thr	Cys	Thr	Glu	Thr	Pro	Leu	Val	Ile	Ala	Gly	Asn	Pro	470
GCT	TAC	CGC	AGC	TTC	AGC	AAC	TCC	CTG	AGC	CAG	TCA	CCG	TGT	CCC	1455
Ala	Tyr	Arg	Ser	Phe	Ser	Asn	Ser	Leu	Ser	Gln	Ser	Pro	Cys	Pro	485
AGA	GAG	CTG	GGT	CCA	GAC	CCA	CTG	CTG	GCC	AGA	CAC	CTG	GAG	GAA	1500
Arg	Glu	Leu	Gly	Pro	Asp	Pro	Leu	Leu	Ala	Arg	His	Leu	Glu	Glu	500
GTA	GAA	CCC	GAG	ATG	CCC	TGT	GTC	CCC	CAG	CTC	TCT	GAG	CCA	ACC	1545
Val	Glu	Pro	Glu	Met	Pro	Cys	Val	Pro	Gln	Leu	Ser	Glu	Pro	Thr	515

FIGURE 4C

ACT	GTG	CCC	CAA	CCT	GAG	CCA	GAA	ACC	TGG	GAG	CAG	ATC	CTC	CGC	1590
Thr	Val	Pro	Gln	Pro	Glu	Pro	Glu	Thr	Trp	Glu	Gln	Ile	Leu	Arg	530
CGA	AAT	GTC	CTC	CAG	CAT	GGG	GCA	GCT	GCA	GCC	CCC	GTC	TCG	GCC	1635
Arg	Asn	Val	Leu	Gln	His	Gly	Ala	Ala	Ala	Ala	Pro	Val	Ser	Ala	545
CCC	ACC	AGT	GGC	TAT	CAG	GAG	TTT	GTA	CAT	GCG	GTG	GAG	CAG	GGT	1680
Pro	Thr	Ser	Gly	Tyr	Gln	Glu	Phe	Val	His	Ala	Val	Glu	Gln	Gly	560
GGC	ACC	CAG	GCC	AGT	GCG	GTG	GTG	GGC	TTG	GGT	CCC	CCA	GGA	GAG	1725
Gly	Thr	Gln	Ala	Ser	Ala	Val	Val	Gly	Leu	Gly	Pro	Pro	Gly	Glu	575
GCT	GGT	TAC	AAG	GCC	TTC	TCA	AGC	CTG	CTT	GCC	AGC	AGT	GCT	GTG	1770
Ala	Gly	Tyr	Lys	Ala	Phe	Ser	Ser	Leu	Leu	Ala	Ser	Ser	Ala	Val	590
TCC	CCA	GAG	AAA	TGT	GGG	TTT	GGG	GCT	AGC	AGT	GGG	GAA	GAG	GGG	1815
Ser	Pro	Glu	Lys	Cys	Gly	Phe	Gly	Ala	Ser	Ser	Gly	Glu	Glu	Gly	605
TAT	AAG	CCT	TTC	CAA	GAC	CTC	ATT	CCT	GGC	TGC	CCT	GGG	GAC	CCT	1860
Tyr	Lys	Pro	Phe	Gln	Asp	Leu	Ile	Pro	Gly	Cys	Pro	Gly	Asp	Pro	620
GCC	CCA	GTC	CCT	GTC	CCC	TTG	TTC	ACC	TTT	GGA	CTG	GAC	AGG	GAG	1905
Ala	Pro	Val	Pro	Val	Pro	Leu	Phe	Thr	Phe	Gly	Leu	Asp	Arg	Glu	635
CCA	CCT	CGC	AGT	CCG	CAG	AGC	TCA	CAT	CTC	CCA	AGC	AGC	TCC	CCA	1950
Pro	Pro	Arg	Ser	Pro	Gln	Ser	Ser	His	Leu	Pro	Ser	Ser	Ser	Pro	650
GAG	CAC	CTG	GGT	CTG	GAG	CCG	GGG	GAA	AAG	GTA	GAG	GAC	ATG	CCA	1995
Glu	His	Leu	Gly	Leu	Glu	Pro	Gly	Glu	Lys	Val	Glu	Asp	Met	Pro	665
AAG	CCC	CCA	CTT	CCC	CAG	GAG	CAG	GCC	ACA	GAC	CCC	CTT	GTG	GAC	2040
Lys	Pro	Pro	Leu	Pro	Gln	Glu	Gln	Ala	Thr	Asp	Pro	Leu	Val	Asp	680
AGC	CTG	GGC	AGT	GGC	ATT	GTC	TAC	TCA	GCC	CTT	ACC	TGC	CAC	CTG	2085
Ser	Leu	Gly	Ser	Gly	Ile	Val	Tyr	Ser	Ala	Leu	Thr	Cys	His	Leu	695
TGC	GGC	CAC	CTG	AAA	CAG	TGT	CAT	GGC	CAG	GAG	GAT	GGT	GGC	CAG	2130
Cys	Gly	His	Leu	Lys	Gln	Cys	His	Gly	Gln	Glu	Asp	Gly	Gly	Gln	710
ACC	CCT	GTC	ATG	GCC	AGT	CCT	TGC	TGT	GGC	TGC	TGC	TGT	GGA	GAC	2175
Thr	Pro	Val	Met	Ala	Ser	Pro	Cys	Cys	Gly	Cys	Cys	Cys	Gly	Asp	725
AGG	TCC	TCG	CCC	CCT	ACA	ACC	CCC	CTG	AGG	GCC	CCA	GAC	CCC	TCT	2220
Arg	Ser	Ser	Pro	Pro	Thr	Thr	Pro	Leu	Arg	Ala	Pro	Asp	Pro	Ser	740
CCA	GGT	GGG	GTT	CCA	CTG	GAG	GCC	AGT	CTG	TGT	CCG	GCC	TCC	CTG	2265
Pro	Gly	Gly	Val	Pro	Leu	Glu	Ala	Ser	Leu	Cys	Pro	Ala	Ser	Leu	755
GCA	CCC	TCG	GGC	ATC	TCA	GAG	AAG	AGT	AAA	TCC	TCA	TCA	TCC	TTC	2310
Ala	Pro	Ser	Gly	Ile	Ser	Glu	Lys	Ser	Lys	Ser	Ser	Ser	Ser	Phe	770
CAT	CCT	GCC	CCT	GGC	AAT	GCT	CAG	AGC	TCA	AGC	CAG	ACC	CCC	AAA	2355
His	Pro	Ala	Pro	Gly	Asn	Ala	Gln	Ser	Ser	Ser	Gln	Thr	Pro	Lys	785
ATC	GTG	AAC	TTT	GTC	TCC	GTG	GGA	CCC	ACA	TAC	ATG	AGG	GTC	TCT	2400
Ile	Val	Asn	Phe	Val	Ser	Val	Gly	Pro	Thr	Tyr	Met	Arg	Val	Ser	800

1	MGWLCSGLLFPVSCVLVLLQVASSGNMKVLQEP TCVSDYMSISTCEWKMG	50
1	MGR LCTKFLTSVGCLILLLV TGS GS I KVLGEPTCFSDYIRTSTCEWF LDS	50
51	PTNCSTELRLLYQLVFL . LSEAHTCIPENNGGAGCVCHLLMDDVVSADNY	99
51	AVDCSSQLCLHYRLMFFEFSEN LTCIPRNSASTVCVCHMEMNRPVQSDRY	100
100	TLDLWAGQQLLWKGSFKPSEHV KPRAPGNLT VHTNVSDTLLLTWSNPYP	149
101	Q MELWAEHRQLWQGSFSPSGNVKPLAPDNLT LHTNVSD EWL LTWN NLYPS	150
150	DNYLYNHLTYAVNIWSENDP ADFRIYNVTYLEPSLRIAAS TLKSGISYRA	199
151	NNLLYKDLISMVNISREDNPAEFIVYNVTYKEPRLSFPINILMSGVYYTA	200
200	RVRAWAQCYN TTWSEWSPSTKWHNSYREFFEQHLLGVSVSCIVILAVCL	249
201	RVRVRSQILTGTWSEWSPSITWYNHFQLPLIQRLPLGVTISCLCIPFLCL	250
250	LCYVSITKIKKEWWDQIPNPARSRLVAIIQDAQGSQWEKRSRGQEPAC	299
251	FCYFSITKIKKIWWDQIPTPARSPLVAIIQDAQVPLWDKQTRSQUESTKY	300
300	PHWKNCLTKLLPCFLEHNMKRDEDPHKA AKEMPFQSGSKSAWCPVEISK	349
301	PHWKTCLDKLLPCLLKHRVKKKTDFPKAAPTKSLQSPGKAGWCPMEVSRT	350
350	VLWPE . .SISVVRVCVELFEAPVECEEEEEVEEEKGSFCASPES SRD .DFQ	396
351	VLWPENVS SVVR CMELFEAPVQNV EEEDEIVKEDLSMSPENSGGCGFQ	400
397	EGREGIVARLTESLFDLLGEENG GF CQDDMGESCLLP SGSTSAHMPWD	446
401	ESQADIMARLTENLFSDDL EAENGGLGQSALAESCSPLPSGSGQASVSWA	450
447	EFPSAGPKEAPPWGKEQPLHLEPSPPASPTQSPDNLTCTETPLVIAGNPA	496
451	CLPMGPSEEATCQVTEQPSHPGP . LSGSPAQSAPTLACTQVPLVLADNPA	499
497	YRSFSNSLSQSPCPRELGPDP LLARHLEEVEPEMPCVPQLSEPTTVPOPE	546
500	YRSFSDCCSPAPNP GELAPEQQQADHLEEEEPSPADPHSSGP . . .PMQP	546
547	PETWEQILRRNVLQHGA AAAPVSAPTS GYQEFVHAVEQGGTQASAVVGLG	596
547	VESWEQILHMSVLQHGAAGSTPAPAGGYQEFVQAVKQGA AQDPGVPGVR	596
597	PPGEAGYKAFSSLLASSAVSPBKCGFGASSGEEGYKPFQDLIPGCPGDP A	646
597	PSGDPGYKAFSSLLSSNGIRGDTAAAGTDDGHGGYKPFQNPVP . . . .NQS	642

FIGURE 5B

647 PVPVPLFTFGLDREPPRSPQSSHLPSSSPÉHLGLEPGEKVÉDMPKPPLPQ 696  
| | | | | | | | | | | | | | | | | | | | | |  
643 PSSVPLFTFGLDTELSPSPINSDPPKSPPECLGLELGLKGGDWVKAPPPA 692  
697 EQATDPLVDSLGSIVYSALTCHLCGHLKQCHGQEDGGQTPVMASPCCGC 746  
| | | | | | | | | | | | | | | | | | | | | |  
693 DQVPKPFDDLGFIVYSSLTCHLCGHLKQHHSQEEGGQSPIVASPGCGC 742  
747 CCGDRSSPPTTPLRAPDPSPGGVPLEASLCPASLAPSGISEKSKSSSSFH 796  
| | | | | | | | | | | | | | | | | | | | | |  
743 CYDRSPSLGSLSGALESCPEGIPPEANLMSAPKTPSNLSGEGK..... 786  
797 PAPGNAQSSQTPKIVNFVSVGPTYMRVS 825  
| | | | | | | | | | | | | | | | | | | | | |  
787 .GPGHSPVPSQTTE....VPVGALGIAVS 810



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

EP 89 31 1244

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	IMMUNOLOGY, vol. 54, 1985, pages 745-754; R.A. DE MAAGD et al.: "The human thymus microenvironment: heterogeneity detected by monoclonal anti-epithelial cell antibodies" * Whole document *	27	C 12 N 15/12 C 12 N 15/62 C 12 P 21/02 A 61 K 37/02 C 07 K 13/00 C 12 P 21/08 G 01 N 33/68
P,X	IMMUNOLOGY, vol. 65, 1988, pages 617-622; M. LARCHE et al.: "functional evidence for a monoclonal antibody that binds to the human IL-4 receptor" * Whole document *	27	
P,X	IMMUNOLOGY, vol. 64, 1988, pages 101-105; M. LARCHE et al.: "A novel T-lymphocyte molecule that may function in the induction of self-tolerance and MHC-restriction within the human thymic microenvironment" * Whole document *	11,13, 27	
P,X	WO-A-8 909 621 (M.A. RITTER et al.) * Whole document *	11,13	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A	PROC. NATL. ACAD. SCI. USA, vol. 84, March 1987, pages 1669-1673; L.S. PARK et al.: "Characterization of the high-affinity cell-surface receptor for murine B-cell-stimulating factor I" * Whole document *	11,13	C 12 N C 12 P A 61 K C 07 K
A	NATURE, vol. 325, 5th February 1987, pages 537-540; J. OHARA et al.: "Receptors for B-cell stimulatory factor-1 expressed on cells of haematopoietic lineage" * Whole document *	11,13	
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>19-01-1990</b>	Examiner <b>DESCAMPS J.A.</b>
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p>			